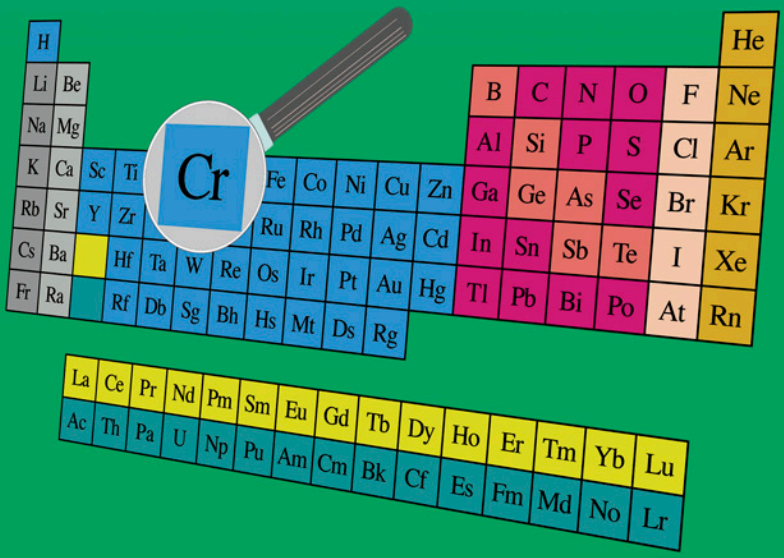
# The Nutritional Biochemistry of Chromium(III)




John B. Vincent
(Editor)

# The Nutritional Biochemistry of Chromium(III)

This page intentionally left blank

# The Nutritional Biochemistry of Chromium(III)

*Editor*

***John B. Vincent***
*Department of Chemistry*
*The University of Alabama*
*Tuscaloosa, USA*

ELSEVIER

Amsterdam • Boston • Heidelberg • London • New York • Oxford • Paris
San Diego • San Francisco • Singapore • Sydney • Tokyo

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

First edition 2007



**Library of Congress Cataloging-in-Publication Data**
A catalog record for this book is available from the Library of Congress

**British Library Cataloguing in Publication Data**
A catalogue record for this book is available from the British Library

ISBN-13: 978-0-444-53071-4
ISBN-10: 0-444-53071-1



Printed and bound in The Netherlands

07 08 09 10 11 10 9 8 7 6 5 4 3 2 1

# Table of Contents

# Preface

The manufacture and sale of chromium nutritional supplements has become a multimillion-dollar industry; these supplements are available in a variety of forms for human consumption including pills, sports drinks, smoothies, and chewing gums and have been recommended for use under certain conditions as supplements in cattle and swine diets. In 1999, sales of products containing chromium reached almost half-a-billion dollars, making the supplements second in sales only to calcium among mineral supplements. Popular interest in chromium(III) is large, as evidenced by numerous recent articles in women's magazines and fitness and health magazines as one might expect for a supplement which has been touted to reduce fat and build muscle without exercise.

Since the 1960s, chromium has been generally believed to be essential for proper carbohydrate and lipid metabolism. Chromium deficiency has been suggested to lead to symptoms associated with adult-onset diabetes and cardiovascular disease, and these supplements have recently found potential as therapeutic agents in the treatment of adult-onset diabetes. However, since the mid-1990s, the long-standing belief that chromium as chromium(III) is essential for humans and other mammals has been seriously questioned, especially since how chromium might function in the body has not been firmly established. The recent lowering of the suggested adequate daily intake of chromium by the National Academies of Science has been questioned by the supplement industry. While the original proposals for a biological role for chromium(III) at molecular levels have been discredited, recent proposals have appeared including a role enhancing insulin receptor's action in the insulin-signaling pathway and that chromium(III) gets converted to the carcinogen chromate (of *Erin Brokovich* fame), which is the biologically active agent. However, the recent research is not without its own questions.

While the benefits of chromium(III) supplementation of healthy humans (e.g., weight loss and increased muscle mass) have all but been discredited since 2001, the use of pharmacological, rather than nutritional, doses of chromium may have beneficial effects on type II diabetes, cardiovascular diseases, and related conditions. The use of these large doses of chromium requires that the toxicological properties of chromium supplements be established. Starting with reports in 1995 that chromium picolinate could have deleterious effects, numerous studies have appeared on potential effects from chromium picolinate and other chromium supplements. Deciphering this literature can

be difficult for the non-expert, especially given attacks by the supplement industry on the academic researchers whose results question the safety of the supplements. This has lead to assessments of the safety of chromium picolinate by the National Academies of Science, Food and Nutrition Board (UK) and the Food and Drug Administration. Similarly, the compound is currently being tested for toxic effects by the National Institutes of Health, which at the same time has recently had a funding initiative to look at using chromium supplements as an adjuvant therapy for diabetes.

The aim of this book is to examine the four most controversial areas of chromium nutrition and chemistry: (1) Is chromium an essential element? (2) What biochemical role if any does chromium(III) have in the body? (3) Can large doses of chromium(III) be used to treat type II diabetes, cardiovascular disease, and related medical conditions? and (4) Is the use of chromium(III) supplements of health concern? This is to be accomplished by having chapters in the four areas written by multiple experts in these areas; however, the experts generally voice opinions on both sides of the issue.

In Chapter 1, I and my graduate student Dontarie Stallings (Department of Chemistry and Coalition for Biomolecular Products, the University of Alabama) outline the research on chromium from the time it was first proposed to be an essential element until 1995, a decade before this book project was initiated. Four chapters address the first aim of the book – whether chromium is an essential element. In Chapter 2, Barbara Stoecker (Regents Professor, Department of Nutritional Sciences, Oklahoma State University and a member of the National Academy of Science panel that established the adequate intake (AI) value for chromium) reviews the data used to determine the AI for chromium. In Chapter 3, Dr Diane Stearns (Department of Chemistry and Biochemistry, Northern Arizona University) argues that chromium should not be classified as an essential element. In Chapter 4, Dr Henry C. Lukaski (Assistant Director of the Grand Forks Human Nutrition Center of the USDA) reviews evidence for effects from chromium supplementation on changes in body mass and body composition, while in turn, Dr Merlin Lindemann (Department of Animal and Food Sciences, University of Kentucky) in Chapter 5 describes the effects of chromium supplementation of animal feed.

Two chapters address the second aim – whether chromium has a biochemical role in the body. In Chapter 6, Dr Weiyue Feng (Institute of High Energy Physics, Chinese Academy of Sciences) reviews how chromium is transported in the body and what this might imply about potential functions for chromium; and in Chapter 7, I and my graduate student Randall Bennett review the recent proposals for how chromium might affect carbohydrate and lipid metabolism. The third aim is addressed in Chapter 8 by Dr William Cefalu (Pennington Biomedical Research Center, Louisiana State University), who reviews the potential use of chromium as a therapeutic agent and Debasis Bagchi (Department of Pharmacy Sciences, Creighton University Medical Center) and colleagues in Chapter 9.

Chapters 10–12 comprise the fourth aim and are, thus, focused on whether chromium supplementation could result in any toxic effects, perhaps the hottest and most highly debated area of current chromium research. In Chapter 10, Diane Stearns analyzes the results of in vitro and cell culture studies on the toxicity of chromium(III) compounds, while Peter Lay and colleagues (Centre for Heavy Metals Research, and Centre for Structural Biology and Structural Chemistry, University of Sydney) in Chapter 11

discuss the implications of recent studies that indicate that chromium(III) complexes may be redox active in vivo. Max Costa and graduate student Qingdong Ke (Nelson Institute of Environmental Medicine, New York University School of Medicine) in Chapter 12 survey toxicological studies on chromium(III) compounds.

Finally, Dr Forrest Nielsen, a distinguished nutritionist at the Grand Forks Human Nutrition Center of the USDA but who has not worked directly in the field of chromium nutritional biochemistry, has provided a summary of the arguments in each of the four areas and presented his own conclusions and interpretations in Chapter 13.

John B. Vincent

This page intentionally left blank

## Contributors

**D. Bagchi**
InterHealth Research Center,
Benicia, CA 94510
Department of Pharmacy Sciences,
Creighton University Medical Center,
Omaha, NE 68178

**M. Bagchi**
InterHealth Research Center,
Benicia, CA 94510

**Randall Bennett**
Department of Chemistry and Coalition
for Biomolecular Products,
The University of Alabama,
Tuscaloosa, AL 35487-0336

**William T. Cefalu**
Pennington Biomedical Research Center,
Louisiana State University System,
6400 Perkins Road,
Baton Rouge, LA 70808

**Max Costa**
Nelson Institute of
Environmental Medicine,
New York University School of Medicine,
57 Old Forge Road, Tuxedo, NY 10987

**Weiyue Feng**
Key Laboratory of Nuclear
Analytical Techniques,
Institute of High Energy Physics,
Chinese Academy of Sciences

**Peter A. Lay**
Centre for Heavy Metals Research,
and Centre for Structural Biology
and Structural Chemistry,
School of Chemistry,
University of Sydney,
NSW, 2006, Australia

**Aviva Levina**
Centre for Heavy Metals Research,
and Centre for Structural Biology
and Structural Chemistry,
School of Chemistry,
University of Sydney,
NSW, 2006, Australia

**Merlin D. Lindemann**
Department of Animal and
Food Sciences,
University of Kentucky,
Lexington, 40546-0215

**Henry C. Lukaski**
US Department of Agriculture,
Agricultural Research Service,
Grand Forks Human
Nutrition Research Center,
Grand Forks, ND 58202-9034

**Irma Mulyani**
Centre for Heavy Metals Research,
and Centre for Structural Biology
and Structural Chemistry,

School of Chemistry,
University of Sydney,
NSW, 2006, Australia

**Forrest H. Nielsen**
USDA, ARS, Grand Forks Human
Nutrition Research Center
Grand Forks, ND 58202-9034

**H. G. Preuss**
Georgetown University Medical Center,
Washington, DC 20007

**Qingdong Ke**
Nelson Institute of
Environmental Medicine,
New York University School of Medicine,
57 Old Forge Road, Tuxedo, NY 10987

**Dontarie Stallings**
Department of Chemistry and
Coalition for Biomolecular Products,
The University of Alabama, Tuscaloosa,
AL 35487-0336

**Diane M. Stearns**
Department of Chemistry
and Biochemistry,
Northern Arizona University, Flagstaff,
Arizona 86011-5698

**Barbara J. Stoecker**
Department of Nutritional Sciences,
Oklahoma State University,
Stillwater, OK 74078

**John B. Vincent**
Department of Chemistry and Coalition
for Biomolecular Products,
The University of Alabama,
Tuscaloosa, AL 35487-0336

**S. Zafra-Stone**
InterHealth Research Center
Benicia, CA 94510

*Chapter 1*

# Introduction: A history of chromium studies (1955–1995)

**John B. Vincent*** **and Dontarie Stallings**

Department of Chemistry and Coalition for Biomolecular Products, The University of Alabama, Tuscaloosa, AL 35487-0336

## INTRODUCTION

While the fiftieth anniversary of the proposal that chromium (as the trivalent ion) is an essential trace element for mammals is rapidly approaching, little progress has actually been made in establishing the nutritional requirement for and biochemistry of chromium over these five decades. This is in stark contrast to the advances in knowledge of the nutritional role and biochemistry of other essential trace elements. The transition metals in the third row of the Periodic Table from vanadium to zinc (V, Cr, Mn, Fe, Co, Ni, Cu, and Zn) and also molybdenum and tungsten are generally considered to be essential for some form of life. Currently, for each of these transition elements except chromium, at least one metallobiomolecule has been well characterized in terms of its function, three-dimensional structure, and mode of action. In fact, whether chromium is essential has been questioned since it was first proposed to be essential over four decades ago; the question of essentialness is still debated openly (see Chapters 2 and 3). Certainly that such a basic question still remains unanswered is problematic.

The field of chromium nutrition and chromium biochemistry has had a problematic past. The purpose of this review is to lay out this past carefully with all of its skeletons exposed. Only from this starting point can the advances presented in the following chapters be put into a clearer perspective with the hope that answers to the many basic questions will soon appear. Five years ago one of the authors wrote a major review of the field of chromium biochemistry which appeared in Polyhedron [1]; this chapter represents a reappraisal of the field with 5 years of hindsight, including some appreciable re-evaluation of some key issues.

* The invited author (and primary author) of the chapter.

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

## CHROMIUM(III)

The form of chromium proposed to be biologically important is the trivalent ion, $Cr^{3+}$. The coordination chemistry of this ion is part of the problem in characterizing chromium-containing species in a biological environment. $Cr^{3+}$ possesses a $d^3$ electron configuration and almost exclusively octahedral coordination. The cation is a hard acid and generally binds oxygen-based ligands, although nitrogen-based ligands are also common. With its three 3d electrons in half-occupied $t_2g$ orbitals in an octahedral environment, its complexes are generally substitutionally inert, making a role for the ion in catalysis most unlikely and potential roles in biological systems probably limited to maintaining structural conformations. In aqueous solution with the type of ligands likely to be found in a biological environment (e.g., oxygen-based species such as carboxylates and phosphates), chromic complexes are usually electrochemically inactive. The electronic spectra of chromic complexes are usually devoid of intense features such as charge-transfer bands; this prevents application of resonance Raman spectroscopy as a useful characterization tool. The visible spectra of its octahedral complexes are dominated by two bands, arising from d–d transitions, with extinction coefficients of $10^2$ or less; the complexes of complexes with oxygen-based ligands are generally green or violet, depending on the relative intensity of the two bands. The paramagnetic nature of the spin 3/2 center makes nuclear magnetic resonance (NMR) studies problematic. In fact, the three-dimensional structure is generally required to interpret the NMR spectra of Cr(III) complexes, rather than NMR being a useful aid in structural characterization. An appropriate source for use in Mossbauer spectroscopy of chromium does not exist. Thus, these common bioinorganic probes are of limited utility. Chromium levels in tissues and biological fluids are extremely low, generally within an order of magnitude of the detection limits of current analytical techniques, which was a major factor limiting studies of Cr in tissue and body fluids prior to 1980 (vide infra).

## THE GLUCOSE TOLERANCE FACTOR (GTF) STORY

### The identification of GTF

The field of chromium biochemistry started in 1955 when Mertz and Schwarz fed rats a *Torula* yeast-based diet which resulted in the rats apparently developing impaired glucose tolerance in response to an intravenous glucose load (in addition to previously identified necrotic liver degeneration) [2]. Shortly thereafter a dietary factor (selenium) was discovered which could reverse the liver disorder but not the glucose intolerance; thus, the authors believed they had identified a new dietary requirement absent from the *Torula* yeast-based diet and responsible for the glucose intolerance, which they coined glucose tolerance factor or GTF [3].

These researchers [4] followed their report in 1959 by identifying the active ingredient of "GTF" as $Cr^{3+}$. Inorganic compounds containing Li, Be, B, F, Ti, V, Mn, Co, Ni, Cu, Zn, Ge, As, Se, Br, Rb, Sr, Y, Zr, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Sb, I, Cs, Ba, La, Ce, Ta, W, Os, Ir, Au, Hg, Tl, Bi, Th, and U (200–500 μg/kg body mass) could not restore glucose tolerance, while several inorganic Cr(III) complexes (200 μg Cr/kg body mass) restored glucose tolerance from a $\leq$2.8% per minute rate of

removal of intravenously injected glucose to the approximately 4% rate of control rats. Brewer's yeast and acid-hydrolyzed porcine kidney powder were identified as natural sources of "GTF", and the active (i.e., effective in reversing the inability to handle the glucose load) ingredient could be concentrated from these materials by physical and chemical means [4]. When given by stomach tube (500–1000 μg/kg body mass), the intact materials and the concentrates could restore proper glucose metabolism in rats on the *Torula* yeast-based diet. Although the separation means to isolate "GTF" were not described in detail, "GTF" was found to be water-soluble, extractable with phenol and isobutanol, and absorbable on charcoal and ion-exchange resins.

From the benefit of over 40 years of hindsight, these studies are deeply flawed despite the success of similar studies in identifying other dietary requirements. Unfortunately, for example, the Cr content of the diet was not reported (although the experimental procedures at the time would not have likely produced the correct value). Additionally, the rats were maintained in wire-mess cages, possibly with stainless steel components, allowing the rats to obtain chromium by chewing on these components. Consequently, the actual Cr intake of the rats in these studies is impossible to gauge, putting into great question the suggestion that the rats were Cr deficient. The use of the large amounts of the metal ions is also of concern; as will be discussed in Chapter 9, large doses of chromium may have pharmacological effects, which may or may not be related to any nutritional requirements. These doses are probably about $10^3$ times the typical daily content of a rat's diet. Questions about data handling and the significance of the effect observed from the chromium treatment have also been raised (vide infra).

Considerable confusion in terminology in terms of the use of GTF has arisen. Studies over the last nearly 50 years have more often than not failed to distinguish the difference among the inorganic ion $Cr^{3+}$, Cr(III) complexes, and the biologically active form of chromium (i.e., the naturally occurring biomolecule(s) which has an inherent function when containing bound Cr(III) if such exists), each called GTF. As originally proposed in 1957, GTF is a substance that is involved in maintaining normal glucose, prevents and cures impairment of glucose removal when given in the diet or by stomach tube, and results in impairment of intravenous glucose tolerance when it is deficient in the diet [3]. If one assumes that chromium is related to GTF and follows this definition, then *GTF can only be the chromic ion*, $Cr^{3+}$. In publications soon after 1959, Mertz and Schwarz equated GTF and trivalent chromium [5, 6]. However, thereafter "GTF" takes on a different usage. As chromium must presumably interact with some organic biomolecule(s) to manifest an effect(s) in mammals, attempts were subsequently made to identify this (these) species. Unfortunately, the products of such attempts have also been termed as "GTF". To distinguish between organic Cr(III) complexes termed "GTF" and $Cr^{3+}$ being referred to as GTF, "GTF" when used to indicate an organic Cr(III) ligand complex will be enclosed in quotation marks.

Research just prior to and in the 1960s was consistent with these early results. Continuing studies using rats fed a variety of diets found that rats on some diets in addition to the *Torula* yeast-based diet apparently possessed low glucose removal rates; the glucose removal rates could be improved by addition of "GTF" concentrates. The concentrates were prepared from dried Brewer's yeast, an enzymatic digest of Brewer's yeast, and dried defatted porcine kidney powder [7]. However, the methods of preparation of these materials were not described; while the amounts of the concentrates

added to the diet were given, the amounts of Cr that these concentrates contained were not given. Again the Cr contents of the diet were not reported. This leaves open the possibility that another agent other than chromium found in the concentrates and lacking in the diets could be responsible for the effect. A study with glucose-intolerant rats on the *Torula* yeast diet found that chromic chloride ($Cr(III)Cl_3 6H_2O$ or actually *trans*-dichlorotetraaquachromium(III) chloride dihydrate) dissolved in the drinking water (5 ppm Cr) or as an aqueous solution given intravenously (1 μg Cr/kg body mass) could reverse the condition, emphasizing the apparent association between $Cr^{3+}$ and GTF [8]. An eye lesion was identified as a common symptom of rats fed a diet considered to be Cr deficient (<100 μg Cr/kg) [9].

$Cr^{3+}$ (as a solution of hexaureachromium(III) chloride or a neutralized solution of chrome alum) in the presence of insulin was found in vitro to enhance glucose uptake by epididymal fat tissue of Cr-deficient rats in a concentration-dependent fashion [5]. Similarly, the uptake of galactose by the fat tissue was enhanced by $Cr^{3+}$ (chrome alum) [10], and the production of $CO_2$ by the fat tissue was found to be potentiated by insulin and $Cr^{3+}$ (hexaureachromium(III) chloride) [11]. This led in part to the postulation of an association between chromium and insulin and the conclusion that "GTF" bound to insulin and insulin receptor to form a ternary complex, stimulating insulin's ability to signal cells [12, 13]. Other evidence came from studies of the effects of chromium and insulin on mitochondria [14, 15]. Polarographic studies with *millimolar* concentrations of chromic chloride indicated that chromium forms complexes with insulin and mitochondria [14]; these complexes, however, formed at non-physiological concentrations of $Cr^{3+}$ far above physiological concentrations (as was indicated by the authors), making any relevance to natural systems unlikely. Furthermore, the observation that the combination of insulin and $Cr^{3+}$ (as a chromic chloride solution) causes swelling of rat liver cell mitochondria [15] raises some caution. Hydrated chromic ions or polynuclear complexes of the type $[Cr_x(OH)_y(H_2O)_z]^{n+}$ (which form when chromic ions are dissolved in water at near neutral pH) must form a complex with insulin and/or a component of mitochondria to be the biologically active form of chromium for this study to be physiologically relevant. However, neither appears to be the case (vide infra).

**Brewer's yeast "GTF"**

Nearly all of the attempts to isolate and characterize "GTF" have involved Brewer's yeast, identified as a source of "GTF" in the 1959 study. "GTF" from Brewer's yeast, isolated by "extraction with ethanol and subsequent chromatography on activated charcoal", was shown by Evans, Roginski, and Mertz to bind to insulin [16]. Acetylation of insulin, but not "GTF", impaired binding.

The next attempts at further characterizing isolated Cr complexes from Brewer's yeast were reported by Evans and coworkers in 1973 [17]. Brewer's yeast grown in a $^{51}Cr$-containing media were extracted by stirring for 4 hours with an equal volume butanol/$H_2O$ mixture. The aqueous component containing 90% of $^{51}Cr$ from the isolated yeast was freeze-dried. The powder was extracted with water, and the soluble components were separated by G-25 size exclusion chromatography or ion exchange chromatography. The $^{51}Cr$ was associated with a 400–600 molecular weight, anionic species whose organic component appeared to be synthesized by the yeast. Infrared studies suggested that the material contained a carboxylate functionality, and the material gave a positive ninhydrin

test. The material was also found to be comprised of at least six amino acids tentatively identified as leucine or isoleucine, proline, valine, alanine, serine, and either glutamic or aspartic acid. The material when administered orally to rats (2 ng Cr) resulted in better chromium absorption than when chromic chloride was given [17].

Mertz and coworkers reported the details of the isolation of Brewer's yeast "GTF" in 1977 nearly 20 years after the initial report [18]. Brewer's yeast was extracted with boiling 50% ethanol. The ethanol was removed under vacuum, and the aqueous residual was applied to activated charcoal. Material active in bioassays (vide infra) was eluted from the charcoal with a 1:1 mixture of concentrated ammonia and diethyl ether. After removal of the ammonia and ether under vacuum, the resulting solution was hydrolyzed by refluxing for 18 hours in 5 M HCl. Finally, the HCl was removed under vacuum, the solution was extracted with ether, and the pH of the solution was adjusted to 3. The cationic orange-red material was further purified by ion exchange chromatography [18]. Unfortunately, these incredibly harsh conditions would have destroyed any proteins or peptides or nucleic acids that initially could have been associated with the chromium. Thus, the cases that the form of chromium recovered after the treatment resembles the form in the yeast are remote at best.

The isolated "GTF" possessed a distinct feature at 262 nm in its ultraviolet spectrum, while mass spectral studies (no data presented) indicated the presence of a pyridine moiety [18]. This led to the identification of nicotinic acid as a component of "GTF"; nicotinic acid was sublimed from the material (no experimental information given) and identified by extraction with organic solvents (no data presented). Amino acid analyses indicated the presence of glycine, glutamic acid, and cysteine as well as other amino acids, although the relative amounts were not reported. The results were interpreted to indicate that "GTF" was a complex of Cr, nicotinate, glycine, cysteine, and glutamate.

The properties of "GTF" were compared to those synthetic complex(es) prepared from $Cr^{3+}$ and nicotinic acids and the above-mentioned amino acids [18]. The material was found to possess an approximate composition of 1 Cr:2 nicotinate:2 glycine:1 glutamate:1 cysteine, but these components only accounted for 88% of the material. The synthetic material and the material from Brewer's yeast behaved similarly upon ion exchange and size exclusion chromatography (unfortunately no estimate of molecular weight was given), as the major component of each coeluted. In paper chromatography experiments, the materials gave several bands; only one from each material was active in the bioassays and migrated with the same $R_f$ value. The chromium in these active bands represented 11 and 6% of the total chromium for the synthetic and Brewer's yeast materials, respectively. The synthetic material and the yeast material possessed similar ultraviolet and infrared spectra [18]. Interpretation of this work is nearly impossible. Characterization of bulk materials of which only a tiny minority is active does not allow for clearly deciphering the composition of the active component(s). Thus, one cannot assume that the amino acid analysis of the bulk product reflects the "active" component. Because of the destructive isolation procedure used to obtain the chromium material from Brewer's yeast (that destroyed proteins from which the observed amino acids were probably generated and also contained conditions under which nicotinate could have been chemically generated from other compounds), the nature of the form of chromium in Brewer's yeast was not successfully determined. The orange-red color associated with both materials is also notable and could suggest that ammonia is present as a chromium ligand.

Based on this work, "GTF" has been proposed to be a Cr(III)–glutathione–nicotinate complex as glutathione is a tripeptide of glutamate, glycine, and cysteine; synthetic complexes made from the combination of $Cr^{3+}$, nicotinate, and glutathione have been reported to have similar biological activity to Brewer's yeast "GTF" and bind tightly to insulin [19]. A three-dimensional structure has also been proposed for Brewer's yeast "GTF" which has two *trans* N-bound nicotinic acid ligands and amino acids occupying the remaining four sites of an octahedral around the chromic center [13]. This proposal, which has been reiterated numerous times in reviews and textbooks as the structure of the biologically active form of chromium, "GTF", has absolutely no basis.

The results of the 1977 study of Mertz and coworkers have not been reproduced in several laboratories [20–24], although conditions varied in many of the later studies. Kumpulainen and coworkers [20] extracted Brewer's yeast with 50% ethanol, removed lipids by extraction with petroleum ether, and then isolated Cr-containing species by size exclusion, ion exchange, and thin-layer chromatography. The material isolated by Mertz and coworkers could not be found. None of the isolated components, whether anionic or cationic, bound insulin.

Mirsky and coworkers isolated an yellow, cationic chromium-containing species from commercial yeast extract powder [24]. The powder was initially extracted with a 1:1 butanol–$H_2O$ mixture before being subjected to dialysis and DEAE-cellulose and Dowex $50W \times 8$ chromatography. The material had a molecular weight below 3500, an ultraviolet absorption maximum at 262 nm, and stimulated fermentation by three strains of yeast. It was also assumed to be the yeast "GTF" reported in 1977 by Mertz and coworkers. No other efforts to determine the composition of the material were reported. Subsequently, this material was shown to affect fermentation by facilitating glucose uptake [25]. The material also reportedly increased decarboxylation of pyruvate by yeast [26] and decreased blood glucose and fatty acids in rats with streptozotocin-induced diabetes without addition of exogenous insulin [27]. The "GTF" also apparently increased 2-deoxyglucose incorporation and increased growth in the yeast *Saccharomyces cerevisiae* in a fashion similar to mammalian insulin, leading to a proposal of a common mechanism regulating metabolic processes in yeast and mammals [28, 29]. Recent studies support the possibility that this yeast shares some parts of the signaling cascades regulating oxidative and non-oxidative glucose metabolism in response to glucose and insulin with insulin-sensitive mammalian cells [30–32].

Gonzalez-Vergara and associates also attempted to isolate "GTF" from Brewer's yeast [21]. A single Cr-containing fraction was found; it was yellow but possessed an ultraviolet spectrum distinct from nicotinic acid. Spectral characteristics suggested the presence of tryptophan or a derivative; the authors concluded that "GTF" is not a Cr–nicotinate complex. A follow-up study [22] found that a species with activity in the rat adipocyte assay for biological activity could be separated from the chromium-containing species. The chromium species possessed Cr(III) in a pseudo-octahedral environment of oxygen (possibly from phosphate ligands) and a molecular weight of 900 and was anionic. The active material possessed an ultraviolet feature at 254 nm and little more than the background level of chromium. The authors concluded that the effect of Brewer's yeast on insulin action in adipocytes might not necessarily be due to Cr-containing species.

Another attempt to identify "GTF" from Brewer's yeast separated 11 apparently homogeneous Cr-containing species from the yeast [23]. The species were amphoteric, anionic, and cationic. The four cationic species and one anionic species displayed some ability to stimulate yeast fermentation. Further investigation [33], however, revealed that all 11 species were artifacts formed between components of the growth media and chromic ions added to the media. The authors proposed that "GTF" could no longer be regarded as a chromium complex.

Holdsworth, Neville, and coworkers have examined Brewer's yeast extracts extensively [34–37]. They too have been able to distinguish between Cr-containing fractions (either cationic or anionic) and a fraction active in in vitro biological activity assays using rat adipocytes; rat hepatocytes from control rats, Cr-deficient rats, and rats on Cr-deficient diets supplemented with chromium and yeast. They conclude that the enhanced response in the presence of insulin comes from gamma-aminobutyric acid in the yeast extracts. No evidence was found that "GTF" aided the binding of insulin-to-insulin receptor, such as by the formation of a ternary complex. The "GTF"-like activity from non-Cr-containing fractions using the adipocyte assay required the cells to be from rats on a *Torula* yeast diet.

Most recently, Hwang and coworkers [38] and also Simonoff et al. [39] showed that the fractions of Brewer's yeast that stimulate glucose oxidation by rat adipocytes in the presence of insulin were distinct from the fractions containing chromium. The rats were raised on a standard (i.e., Cradequate) diet.

**Biological activity assays**

Until this point in time, biological activity of a chromium-containing species referred to the ability of the species to potentiate the action of insulin to stimulate in vitro the metabolism of epididymal fat tissue from "chromium-deficient" rats. "Chromium-deficient" rats in this definition means rats on the *Torula* yeast diet or another diet giving rise to similar effects when rats are given glucose tolerance tests (which, as discussed earlier, have essentially no data supporting their actually being chromium deficient). The reproducibility and sensitivity of the assay for biological activity was improved in 1978 by Anderson and coworkers who showed that adipocytes isolated from epididymal fat tissue of "Cr-deficient" rats could be utilized [40]. Replicate assays at a series of insulin concentrations could now be readily performed, allowing for detailed kinetics experiments. These assays showed that Brewer's yeast extracts and the synthetic Cr–nicotinate complexes described above potentiated the ability of insulin to stimulate glucose oxidation at a variety of insulin concentrations. The degree of stimulation also depended on the chromium concentrations of either the extract or synthetic complexes, leading to the conclusion that these compounds contain biologically active forms of chromium. However, further analysis of these results has shown that the original interpretation is incorrect [41]. Addition of the chromium sources without adding insulin stimulated the metabolism of glucose; the stimulation due to insulin above this increased background was actually decreased. The chromium sources in fact made insulin less effective, consistent with components of the extracts binding to insulin (as described above) and not allowing the complexed insulin to bind to its receptor. An alternative has been pointed out in that the isolated adipocytes may not have been washed sufficiently to remove traces of insulin [42]; however, extrapolation of the insulin dose

response curves indicates that insulin levels would have been too far to high in these preparations for this to be possible.

The use of the microbiological assay (yeast fermentation assay) to determine biological activity is not only interesting but also problematic. The yeast fermentation assay reportedly yields results that parallel rat fat pad assays when using Brewer's yeast "GTF" as a Cr source [43]. However, as the active component of yeast extracts can be separated from chromium, what exactly this assay measures in regard to chromium is questionable.

**Porcine kidney powder "GTF"**

The other source of GTF in addition to Brewer's yeast originally proposed by Schwarz and Mertz [4] was porcine kidney powder, which has received much less attention. In the only report on isolating "GTF" from porcine kidney or kidney powder after 1960, Haylock and coworkers identified one cationic chromium-containing species from kidney powder [23]. The species was isolated and concentrated by ion exchange chromatography (without a hydrolysis step). The material was extremely active in "biological activity" assays that used the rate of fermentation of chromium-deficient yeast. Although reported after 1995, another result needs to be mentioned here; an oligopeptide named low-molecular-weight chromium-binding substance (LMWCr) (vide infra) has been isolated from porcine kidney and porcine kidney powder [44]; hydrolysis of this material produces species similar in properties to "GTF's" from Brewer's yeast and kidney powder, suggesting the original reports of these "GTF's" are on artifacts.

**Other questions regarding "GTF"**

The reproducibility and interpretation of other results associated with research on "GTF" have been questioned. Woolliscroft and Barbosa have examined the effects of a normal and a *Torula* yeast diet in intravenous glucose tolerance tests in rats [45]. They reproduced the results of Mertz and Schwarz; yet, observation of a significant difference in glucose metabolism between the two groups of rats depended on the method used to present the data, that is using total plasma glucose concentrations versus using excess plasma glucose concentrations. (Excess plasma glucose concentration refers to the total plasma glucose concentration minus the fasting plasma glucose concentration.) The effect was only statistically significant when excess plasma glucose was used. As calculating the excess plasma introduces error (this error was not considered in the statistical analyses), use of actual measured plasma glucose is the accepted practice. This places the observations by Schwarz and Mertz of an effect from the *Torula* yeast diet on glucose clearance rates into serious doubt.

Shepherd and coworkers examined the in vitro production of carbon dioxide and fatty acids from glucose by adipocytes from rats on a control diet or diets using either *Torula* yeast, Brewer's yeast, or casein as the sole protein source [46]. Glucose utilization increased only for fat cells from rats fed the *Torula* yeast diet when the material isolated from Brewer's yeast, as described by Mertz and coworkers in 1977 [18], was added to the assay. The levels of amino acids and 22 of 23 trace elements were comparable in all diets. Manganese content was low in all diets but especially low in the *Torula* yeast diet. The authors proposed that Mn deficiency might be responsible for the effects of the *Torula* yeast diet. This suggestion would appear to be contradicted by the results of

the study by Schwarz and Mertz in 1959 which observed no effect on "Cr-deficient" rats when the diet was supplemented by Mn [4]. The *Torula* yeast diet of Shepherd and coworkers possessed 0.6 μg Cr/g dry mass of diet [46]. A *Torula* yeast diet used by Mertz, Anderson, and coworkers has been reported to contain no greater than 0.040 μg Cr/g dry mass [41], a difference in Cr content of greater than 15-fold. Irregular intakes of minerals other than chromium can lead to changes in glucose tolerance or insulin resistance and can be a concern in experimental diets. Thus, this emphasizes the problem of the lack of knowledge of the Cr content of the original *Torula* yeast diet of Schwarz and Mertz.

### Conclusions

Over four decades ago, Schwarz and Mertz reported that $Cr^{3+}$ (or GTF) is a nutrient for mammals; inorganic Cr(III) complexes apparently could restore the glucose tolerance of rats fed a *Torula* yeast-based (supposedly Cr-deficient) diet. Major portions of these studies, which were considered the pioneering work in the field of chromium biochemistry and nutrition, have been effectively refuted. The diet used by these workers has not been demonstrated to be chromium deficient; thus, any effects from supplementing the diet with quantities of Cr several fold larger than the normal dietary intake does not establish an essential requirement. Similarly, proposals that Cr acts by binding to insulin are without foundation.

*The biologically active form of chromium is not "GTF", reportedly a Cr(III)–nicotinate–amino acid (or glutathione) complex.* In fact, the composition of the artifact from yeast isolated by Mertz and coworkers was actually not established, and proposals for the three-dimensional structure of "GTF" are simply science fiction. The consensus is that the effects of materials isolated from Brewer's yeast observed by Mertz and coworkers [18] were probably serendipitous, as $Cr^{3+}$ has been demonstrated repeatedly to be separable from agents in yeast responsible for in vitro stimulation of glucose metabolism in adipocytes. Yet, even the detailed results of the most recent studies of yeast "GTF" by different laboratories still cannot be reconciled completely. *Given the considerable confusion over the names GTF and "GTF" and the history of the quest for "GTF", the author recommends that the use of the term glucose tolerance factor or GTF (and "GTF") be terminated.*

One unexplained item related to these studies is the accumulation of chromium by Brewer's yeast. The yeast presumably accumulate chromium for a reason. The reasons for the accumulation, including that fermentation by the yeast may be accelerated by chromium, are interesting bioinorganic questions worthy of a systematic study and potentially could reconcile or explain some of the results described above.

## THE RACE TO SYNTHESIZE A MODEL OF "GTF"

The proposed identification of nicotinate (3-carboxypyridine) in "GTF" stimulated an interest in the synthesis of chromic-nicotinate or -nicotinic acid ester complexes starting in 1981 [47–57]. (For a review see reference 58.) However, the inability of the biologically active form of chromium to be elucidated (i.e., the problems with the isolation of Brewer's yeast "GTF") and lack of success of attempts to synthesize and characterize

the synthetic "GTF" of Mertz and coworkers [18] led to a rapid decline in studies after 1985. In well-characterized complexes, nicotinate coordinates in a variety of manners to Cr(III): bridging through the carboxylate (μ-O,O') [48], monodentate through a carboxylate oxygen [51], or monodentate through the pyridine nitrogen [56]. Legg and coworkers developed a clever method for determining whether nicotinate (either as anion or as zwitterion) is bound through the oxygen(s) or nitrogen using $^2$H NMR [53, 56, 59]. The $^2$H NMR signal of 2-d-nicotinate compounds is shifted to approximately −70 to −75 ppm if the ligand is bound through the nitrogen and to +6 to +10 ppm if bound through the oxygen. Recently, all the nicotinate proton or deuteron resonances of a number of Cr(III)–nicotinate complexes have been assigned [60]. (The assignment for Cr(III)–nicotinate complexes described by Broadhurst et al. [61] are incorrect as no paramagnetically broadened resonances were observed [60].)

A number of studies have examined a material characterized as "Cr(nicotinate)$_2$(OH)(H$_2$O)$_3$" (subsequently to be referred to as chromium nicotinate or chromium polynicotinate for consistency with the nutrition literature), which is the product of the reaction of two or three equivalents of nicotinic acid with chromic ions in aqueous solution at elevated temperatures [52, 60–63]. The complex has not yet been crystallized, and NMR studies suggest that the proposed formula would indicate it is more complex [60]. The original interest in the complex arose from its ability to stimulate $CO_2$ production in Cr-deficient yeast [52, 54]. However, the problems with this assay were described above. Because this chromium–nicotinate material is absorbed better than dietary chromium by mammals [62, 64], it has gained substantial use as a nutritional supplement, especially before the rise in the popularity of chromium picolinate.

## THE CHROMIUM PICOLINATE (Cr(pic)$_3$) STORY

### Nutritional supplement?

In addition to attempts to synthesize and characterize Cr(III)–nicotinate complexes, the products of reaction of Cr(III) sources and the related pyridinecarboxylic acids picolinic acid (2-carboxypyridine) and isonicotinic acid (4-carboxypyridine) [65] have also been studied in some detail. Chromium(III) picolinate, Cr(pic)$_3$, has been the most thoroughly studied of these synthetic products and has become a very popular nutritional supplement; products containing Cr(pic)$_3$ generate over one hundred million dollars in sales annually as the supplement is available over-the-counter in numerous forms including pills, chewing gums, sports drinks, and nutrition bars. Cr(pic)$_3$ is a relatively well absorbed form of chromium (~2% efficiency compared to dietary chromium which is only absorbed with approximately 0.5% efficiency) [62, 64]. (This degree of absorption or "bioavailability" versus dietary chromium or inorganic chromic salts is not unique and is shared by other organic Cr(III) ligand-complexes such as "Cr(nicotinate)$_2$(OH)(H$_2$O)$_3$") [62, 64]. Cr(pic)$_3$ has been proposed to be the biologically active form of chromium [66]; yet, Cr(pic)$_3$ has been shown neither to possess intrinsic biological activity nor to occur naturally in mammals or other organisms, and no reason exists to expect it to exist naturally in vivo, especially as chromium and picolinic acid levels in tissues make its generation unlikely at best.

The study of the effects of chromium picolinate on mammals has been extremely contentious. This has recently been stated well in a review by Hellerstein [67]:

To an outsider reviewing literature on chromium and diabetes/obesity, the field is most striking for two features: its nearly complete lack of biomedical or clinical understanding and its high degree of polarization . . .. As in all fields with more heat than light, the reason has been the incomplete ability to measure and test key factors . . .. The high degree of politicization and polarization in this field is characterized by unproven claims and counterclaims and suspicion among investigators. Concerns about possible commercial bias and potential conflict of interest have naturally emerged. Reports of benefits of supplementation (e.g., that lean tissue is increased and fat decreased by chromium in athletes in training) [68] that were not confirmed by several subsequent studies [69–71] have furthered these concerns.

The first nutritional, biochemical, or medicinal studies with $Cr(pic)_3$ were reported in 1989 [68]. Evans found that in double-blind, crossover studies volunteers taking 200 μg/day chromium as $Cr(pic)_3$ (compared to taking placebos) had decreased total cholesterol, LDL cholesterol, and apolipoprotein B and increased apolipoprotein A1 [68–72]. In a similarly designed study, eight of eleven adult-onset diabetic patients displayed positive effects with chromium supplementation (vide infra). Two studies by Evans with young men participating in weight training resulted in significant losses in body fat and increases in lean muscle mass with chromium supplementation [68]. These human studies were followed by studies with rats and humans [63, 66, 73]. Studies of cultured rat skeletal muscle myoblasts found increased insulin internalization and accompanying glucose and leucine uptake by cells in media containing 1 μM $Cr(pic)_3$ but not containing the same concentration of chromic chloride, chromium nicotinate, or zinc picolinate [66]. The results were postulated to result from increased membrane fluidity. Rats fed $Cr(pic)_3$, but not rats fed chromium nicotinate (1 μg Cr/g diet for 200 days), had decreased plasma glucose and glycated hemoglobin [63]. In another study, similar results were observed after 1000 days on the diet; also 80% of rats on $Cr(pic)_3$ were alive after 41 months, while all rats fed equivalent amounts of chromium nicotinate or chromium chloride had died [73].

The results of the first human studies [68, 72] were questioned rapidly by Lefavi [74] and Lefavi, Anderson, et al. [75]; Lefavi pointed out that the reported 4.6-pound lean body mass increase in males and 4.0-pound increase in females in 12 weeks of a weekly aerobics class were "preposterous" and that the studies were poorly controlled and had few subjects [74].

The history leading to the first publication in 1989 has been excellently summarized by Forrest Neilsen of the USDA (Grand Forks Human Nutrition Research Center) [76]. While examining the use of zinc picolinate to treat children with acrodermatitis enteropathica, a genetic disorder that results in the inability to absorb zinc from cow's milk, Gary Evans, employed by the Grand Forks Human Nutrition Research Center, found that zinc and other metal picolinates were absorbed better than the corresponding mineral salts. Evans patented the process of synthesizing coordination complexes of picolinic acid [76]. Nutrition 21 licensed the patent from USDA in 1986 and supported Evans research leading to the 1989 publications. "In other words, contrary to what many advertisements touting chromium picolinate lead many to believe, the USDA patent is not specific to chromium, nor does it mention that chromium picolinate has any beneficial effects claimed for this form of chromium supplement" [76].

This area has received much attention since these initial reports, and effects of chromium nutritional supplements on body composition and body mass are reviewed in Chapter 4.

**Toxic effects?**

In 1995, questions arose about the safety of $Cr(pic)_3$ as a dietary supplement as Wetterhahn and coworkers showed that the compound caused clasotogenic damage in a Chinese hamster ovary (CHO) cell model [77]. When intracellular chromium levels generated using $CrCl_3$ or chromium nicotinate were comparable to those generated using $Cr(pic)_3$, no chromosome aberrations were found. Wetterhahn and coworkers also suggested that taking $Cr(pic)_3$ supplements for 5 years could result in liver tissue concentrations of 13 μM [78]. The investigation of the potential toxic effects of the use of chromium picolinate or other Cr(III) compounds as nutritional supplements or pharmaceuticals has been an active and controversial area of investigation. The toxicology of Cr(III) is addressed in Chapters 10–13.

**Inorganic chemistry**

The inorganic chemistry of chromium picolinate has a long history. The pinkish red crystalline compound is usually prepared by the reaction of simple Cr(III) salts (e.g., chromic acetate [79], chromic nitrate [80], chromic perchlorate [81], chromic chloride [82], or chromic sulfate) [83] and picolinic acid in water following the procedure of Ley and Ficken reported in 1917 [84]. It has also been prepared by the direct reaction of $Cr(OH)_3$ [85] and picolinic acid, the reaction of $Cr(CO)_6$ and the acid in methanol [86], or the oxidation of chromous ions by $[Co(NH_3)_5(pic)]^{2+}$ [87]. The preparation of the complex in water by the method of Ley and Ficken has long been recognized to result in the formation of a purple byproduct of empirical formula "$Cr(III)(pic)_2(OH)$" [79, 88], although it has been misidentified as an isomer of $Cr(pic)_3$ [89]. The byproduct is actually the dinuclear complex $Cr_2(\mu\text{-}OH)_2(pic)_4$, which has been characterized by Stearns and Armstrong [79]. A mixture of monomer and dimer is also formed by the reaction of chromic salts with picolinic acid and aqueous hydroxide in ethanol [88]; the two products can be separated by their solubility in acetonitrile. Unfortunately, this procedure also gives rise to another intractable product, possibly polymeric chromic oxides, and the yields of monomer and dimer have not been reported. Stearns and Armstrong showed that increasing pH results in an increase of the yield of the dimer relative to monomer from aqueous solution, although only a 16% isolated yield of dimer was reported [80]. Recently, a high yield preparation of the dinuclear complex has appeared [90]. The three-dimensional structures of both the mononuclear and the dinuclear complexes have been determined by X-ray crystallography; this technique clearly reveals the red monomer to be the meridinal isomer [80], despite earlier claims based on electronic spectroscopy that it was the facial isomer (reviewed in [80]). The existence of the facial isomer in solution or solid phase has yet to be established.

Measuring the electronic and $^1H$ NMR spectra of $Cr(pic)_3$ has been problematic, but questions about the electronic spectrum have recently been resolved [90]. Because of the low solubility of the complex in water and other common solvents, electronic spectra were often measured in dilute mineral acid solution, in which the complex decomposes with time [80, 90]. Despite an original claim that no $^1H$ NMR signals could be observed

for $Cr(pic)_3$ [61], the paramagnetic $^1H$ and $^2H$ NMR spectra have recently been obtained and assigned [60]. Unfortunately, the low solubility of $Cr(pic)_3$ in $H_2O$ and the broadness of the NMR signals prevent electronic and NMR spectroscopy from being useful in following the complex in biochemical or nutritional studies. The lipophilicity of $Cr(pic)_3$ has been measured and found to be surprisingly small [60], making the proposal that $Cr(pic)_3$ may act by affecting lipid bilayer fluidity [66] highly unlikely.

The redox potential of $Cr(pic)_3$ has recently been reported [91]. The compound is considerably harder to reduce than previous estimates suggested. In DMF, the Cr(II)/Cr(III) couple has an $E_{1/2}$ of –1.23 V vs. normal hydrogen electrode, while the dinuclear compound $Cr_2(\mu\text{-}OH)_4(pic)_4$ is harder to reduce ($E_{1/2} = -1.45$ V) [91]. No features are observed at positive potentials until solvent decomposition occurs for either compound. The potential for the reduction of $Cr(pic)_3$ is slightly shifted in the positive direction in water (A. Pickering and J. Vincent, unpublished results). The implications of this for the toxicology of $Cr(pic)_3$ will be discussed in later chapters.

## PHYSIOLOGICAL STUDIES

Studies of the first two forms of chromium proposed to be biologically active and the two forms most likely to be familiar to bioinorganic chemists, "GTF" and $Cr(pic)_3$, have numerous difficulties associated with them. Neither is the biologically active form of chromium and questions arising from these studies have led to questioning of the original studies by Mertz and Schwarz on the requirement of chromium by mammals. This section of the review re-examines and summarizes the evidence for an essential role for chromic ion through 1995. Focus will be placed on studies with rats and with humans. Rat studies will be a focus because they are fairly numerous and are generally the most carefully controlled studies. Recent studies with humans and rats are discussed in Chapters 2–4 and 8. Studies with other animals are reviewed in Chapter 5.

### Rat

Studies on the effects of diet-induced Cr deficiency and Cr supplementation of rats can be divided into two categories. The distinction lies in how carefully the Cr deficiency and other dietary stresses to aid in the induction of Cr deficiency were defined. This has been well stated by Anderson and coworkers: "It should be noted that the mineral content of the low-Cr *torula* yeast/sucrose diets used in the early studies [2, 3, 7] and in more recent studies [8, 34, 36, 45, 92] on Cr deficiency were not completely defined and differed considerably from diets prepared using purified materials or commercial rat chow. Diets used in the earlier studies resulted in undefined dietary stresses [93]." Studies are also complicated by the lack of an indicator of Cr status in rats other than the effects of chromium supplementation; the threshold dietary chromium concentration between a $Cr^{3+}$-sufficient and a $Cr^{3+}$-deficient diet has not been established.

The first studies by Mertz and Schwarz [4] which used well-characterized Cr(III) complexes utilized rats fed a commercial laboratory chow which was deemed to be "GTF" deficient as it gave rise to reduced glucose removal rates in intravenous tolerance tests as had the *Torula* yeast diet (30% yeast, 59% sucrose, 5% vitamin E-free lard, and vitamins and minerals) [2, 3, 94]. The Cr content of the diet was not reported. Rats

fed the chow had a glucose removal rate of 2.8% (or lower) administered glucose per minute after an intravenous injection of 1250 mg glucose/kg body mass [4]. Significant improvement (even restoration of normal rates, i.e. ~4%) resulted when rats were given several Cr(III) complexes (200 μg Cr/kg body mass) by stomach tube 18 hours before the glucose tolerance tests. Chrome alum was subsequently shown to be effective in increasing excess glucose removal rates in rats on the *Torula* yeast diet when given at levels of 2.5 mg Cr/kg body mass (or greater) by intravenous injection 2 hours before the glucose tolerance tests [11]. The rats were kept in wire mesh cages; the durations of feeding the diets were not given (other than that the diet was used long enough to give the depressed glucose removal rates). Woolliscroft and Barbosa (as described above) gave 6-week-old rats a diet comprised of 30% *Torula* yeast, 50% sucrose, 15% lard without antioxidants, and vitamins and minerals for 6 weeks. For the glucose tolerance tests, rats were given 1250 mg glucose/kg body mass. For comparison with control rats, Cr-supplemented rats were given 5 ppm Cr as chromium chloride in their drinking water over the 6 weeks on the diet. Glucose removal rates varied significantly between the control and supplemented rats only if excess glucose rates were used rather than the more commonly used total glucose removal rates [45].

Schroeder and coworkers have performed similar studies during the 1960s and 1970s. The diet was comprised of 60% seed rye, 30% dried skim milk, 9% corn oil, and 1% NaCl with added vitamins and minerals and contained 100–200 μg Cr/kg. In the first of these studies, rat received 5 ppm Cr in their drinking water. After 360 days of treatment, mortality was low, while growth of male rats, but not that of females, was stimulated [95]. For supplementation in another study, rats were given water containing 2 ppm Cr as chromic acetate [96]. Male and female rats were fed the diet or diet supplemented with chromium for 300–650 days after weaning. Cr-deficient rats had higher fasting (18 hour) serum glucose levels. Other rats were fed the *Torula* yeast diet; Cr-supplemented rats were found to grow more rapidly than rats not supplemented with Cr [96]. Elevated serum glucose was not observed as in the earlier work by Schwarz and Mertz. Mertz and Schroeder collaborated on examining glucose removal rates for rats on the seed rye diet (Cr content ~100 μg/kg wet weight) [8]. Cages were acrylic with stainless steel covers. Rats were fed the diet from weaning until age 456–752 days, then maintained on the *Torula* yeast diet for only 4 days. Pre-supplementation glucose tolerance tests were performed on six rats, and four of these rats were given Cr as chromium chloride (5 ppm) in the drinking water and received an intravenous dose of Cr (1 μg Cr/kg body mass). Glucose tolerance tests were given 2 hours after the injection and again after 4–7 days. Supplemented rats showed significant increases in glucose removal rates [8]. While not stated, excess glucose rates appeared to have been used. Schroeder returned to comparing the results of the *Torula* yeast diet and rye seed diet in 1966 [97]. Rats on both diets possessed higher serum glucose concentrations than rats on the diet supplemented with Cr.

In 1968 and 1969, Schroeder also examined the effects of Cr on cholesterol levels [98, 99]. In the first study, rats from weaning to 11–30 months of age received 1–5 ppm Cr as Cr acetate, while control rats received ~80 μg Cr/kg body mass daily via food and water. While results are somewhat difficult to compare because of the range of number of animals, length of treatment, and amount of Cr provided, male rats on 1 ppm Cr appeared to possess lower serum cholesterol levels, while females required 5 ppm Cr to

suppress cholesterol levels [98]. In the later study, weanling rats were provided a *Torula* yeast diet containing 50% sucrose [99]. A portion of the rats received 5 ppm Cr in their drinking water as Cr acetate. After 318 or 322 days of treatment, male and female rats, respectively, on Cr had lower serum cholesterol levels, while no effect was observed after 151 days for the male rats. Female rats were not tested at the earlier time.

Some other studies before 1970 are notable. Staub and coworkers found that rats on a high cholesterol diet (added cholesterol and cholic acid) with either sucrose or potato starch as the carbohydrate source had higher serum cholesterol concentrations than rats on the diets supplemented with 5000 μg/kg Cr (chromic acetate) in the drinking water [100]. The diet reportedly contained 310 μg/kg Cr (sucrose) or 380 μg/kg Cr(starch). Similar results had been obtained previously by Schroeder and Balassa, but other metals (Cd, Pb) also elicited a similar effect [101]. Roginski and Mertz examined the effects of a high sucrose diet (77% sucrose, 10% soy protein, 8% distilled lard, and vitamins and minerals) [102, 103]. Rats in plastic cages on the low-Cr diet (<100 μg/kg) had moderately depressed growth which could be exacerbated by exercise and had their fasting blood glucose levels decreased more by insulin when compared to supplemented rats (2 ppm Cr in drinking water).

In 1978, O'Flaherty and McCarty reported the use of a purified "low-Cr" diet (800 μg Cr/kg diet) comprised of 62.4% cornstarch, 3.0% cellulose, 20% egg white, and vitamins and minerals [104]. A portion of the rats were supplemented with 5 μg Cr (chromic acetate)/mL drinking water. Supplementation did not affect growth, but rats on the purified diet had higher mean fasting glucose levels. The higher plasma glucose levels were maintained during glucose tolerance tests (after 8 weeks on the diet).

Between 1980 and 1995, few studies on rats were executed. Anderson and Polansky reported that male rats on the *Torula* yeast diet had decreased sperm counts and decreased fertility at 8 months of age [105]. Anderson and coworkers found no change in growth rate for rats given a purified low Cr, high sucrose diet versus that of Cr-supplemented rats (2 ppm Cr in drinking water) [106]. Jain et al. [107] fed rats in plastic cages a diet containing 80 μg Cr/kg diet for 11 weeks. Compared to rats supplemented with Cr by adding chromic acetate to the drinking water (1.140 mg Cr/mL), rats fed the Cr-deficient diet had similar mean body mass and similar responses during intravenous glucose tolerance tests. However, the unsupplemented rats had higher fasting blood glucose. Donaldson, et al. [108] found that rats kept in plastic cages and fed a synthetic high sucrose (68.4%), high cholesterol (2%) diet "low in Cr" (60–100 μg/kg) from weaning until 18 months of age had normal growth. Plasma cholesterol and triglyceride levels in the rats did not differ from those of rats given supplemental chromium (5000 μg Cr as potassium chromium sulfate per kg diet). At age 4 and 8 months, 1-hour postgavage (2500 mg glucose/kg body mass) plasma glucose concentrations were statistically higher in the low-Cr rats, but this difference disappeared at 12 months. Flatt et al. [109] fed rats a "low-Cr diet" (30 μg Cr/kg) or a Cr-containing diet (1000 μg/kg) from weaning to 53 days of age. The diets were high neither in sucrose (<9% sugars) nor in fat (~5%). At 53 days, the rats had similar body masses, food intake, glycosylated hemoglobin levels, and plasma glucose and insulin concentrations. Plasma glucose levels of the rats responded in an identical fashion to intraperitoneal glucose and insulin.

Results of these last two studies have questioned whether the effects of the previous studies were due to the element chromium or due to other dietary factors. Interpretation

of the results of these approximately 35 years of studies is definitely complicated by the variety of experimental conditions. When studies using the problematic yeast diet and studies using analytical techniques that cannot guarantee the accuracy of the Cr contents of the "low-Cr" diets (before *c*. 1980) are eliminated from consideration, out of the studies described above only the studies by Jain et al. [107], Donaldson et al. [108], and Flatt et al. [109] remain. The effects of these "low-Cr" diets (30–100 μg/kg) are equivocal at best. No beneficial effect from Cr supplementation is observed in common between any two of the studies.

Another study appeared at the end of the period of time covered by this review and is probably the most carefully performed of the studies to this time [110]. Rats in plastic cages (with no access to metal components) were given a diet consisting of 55% sucrose, 15% lard, 25% casein, and vitamins and minerals ($33 \pm 14$ μg Cr/kg diet) [110]. The sucrose levels were used to theoretically help to attempt to induce Cr deficiency as dietary carbohydrate stress leads to increased urinary chromium loss (vide infra). To compromise pancreas function, low copper concentrations (1 mg/kg) were employed the first 6 weeks; high dietary iron concentrations were used throughout to potentially aid in obtaining Cr deficiency. A supplemented pool of rats was given water containing 5 ppm $CrCl_3$. Over 24 weeks, body masses were similar for both groups. At 12 weeks, Cr-deficient rats had lower fasting plasma insulin concentrations and similar fasting plasma glucose levels compared to supplemented rats. Both concentrations were similar after 24 weeks. In intravenous glucose tolerance tests after 24 weeks on the diet, plasma insulin levels tended to be higher in Cr-deficient rats; rates of excess glucose clearance were statistically equivalent. Glucose area above basal was reported to be higher in Cr-deficient rats; however, at every time point in the glucose tolerance test, the plasma glucose concentrations of each pool of rats were statistically equivalent, suggesting that the difference in area arises from a mathematical error. Thus, a 55% sucrose diet low in chromium appears to lead to hyperinsulinemia, possibly reflecting defects in peripheral tissue sensitivity to glucose [110]. Do the effects of supplemental Cr correspond to improvement in Cr nutritional status or to alleviation of the effects of the other dietary stresses?

Comparison of these results is now necessary to try to draw any further conclusions. The division, by Anderson, of the rat diets into purified and semipurified (*Torula* yeast and rye seed) diets is useful. The effect on excess glucose clearance rate observed somewhat consistently with the *Torula* yeast diet (which is high in sucrose) is not observed with purified, low-Cr, 55–64% sucrose diets. (Also, the chromium content of this *Torula* yeast diet may be quite variable (vide supra) [46].) This again questions what dietary component gives rise to this effect when using the *Torula* yeast diet. Symptoms of chromium deficiency probably could not have been observed in studies using stainless steel cages (a source of chromium). The effects of growth observed in some studies with the semipurified diets are not observed in more recent studies with the purified diets, also questioning the origins of these effects. Higher serum cholesterol levels observed with one of the semipurified diets are not observed with the purified diets. Thus, the studies are inconsistent so that no effects can conclusively be attributed to Cr deficiency in the rats, if this state was ever achieved.

Whether any of the diets is Cr deficient is an interesting question, as is what the minimum required adequate daily intake of Cr for a rat is. A reasonable answer can

be made based on human studies. Humans appear to lack sign of Cr deficiency with a daily intake of 30 μg Cr (vide infra). Assuming an average body mass of 60 kg, this corresponds to 0.5 μg/kg body mass. Given the typical daily food intake of a rat, this corresponds to rats eating a diet with a dry weight composition of ~3 μg/kg food. Given this, none of the so-called "low-Cr" diets are actually low in Cr, even if rats require ten times the daily quantity of Cr as humans. Thus, these studies apparently provide no data on the effects of chromium deficiency. However, they do provide some conflicting data on the effects of pharmacological doses of Cr on rats under a variety of conditions. Fortunately, better controlled and performed studies on rats in the last 10 years are helping to clarify the situation.

**Human**

The studies with rats indicate the difficulty in generating chromium deficiency; great care – such as feeding purified diets with <100 μg/kg Cr (which is probably far in excess of the amount of Cr needed), carefully controlling the environment including prevention of access to stainless steel, and applying additional dietary stress (e.g., a sucrose-based diet) – is required, while the studies reveal that Cr deficiency may not have yet been reached. Approaching this level of control in most human studies is not possible; thus, large effects from chromium supplementation should not be expected. Fortunately, studies examining the effects of chromium supplementation on human diets have been reviewed several times [111–116]; unfortunately, varying conclusions have been reached. This is based in part on what studies are covered by the reviews. The problems arise from three considerations: difficulties with analytical techniques to determine chromium levels in biological samples, the need to use well-characterized sources of Cr, and the necessity of careful experimental design.

Chromium levels in tissues, food components, and other biological samples reported prior to approximately 1978 are problematic [117, 118]. Improvements in analytical techniques revealed several problems including appreciable contamination of biological samples such as by homogenization in a stainless steel blender; in fact, measured chromium levels reflected the levels of contamination not the actual tissue or fluid Cr concentrations, which are extremely small. Another major problem in atomic absorption experiments was that prior to 1978 workers were attempting to measure a tiny signal against a large background; in fact, a linear correspondence was found to exist between background absorbance and the "apparent Cr content" of samples [117]. Currently, analyses of human blood and urine samples with Cr concentrations above 1 ppb should be considered suspect. Consequently, studies prior to 1978 that utilize patients who are believed to be Cr deficient based on Cr tissue or fluid concentrations and that report Cr levels in tissues or fluids in order to several orders of magnitude too high must be considered questionable at best.

Several early studies of the effects of Cr supplementation of human diets involved the use of Brewer's yeast or Brewer's yeast grown in media with a high chromium content (e.g., [119]). Given the difficulties described above with the yeast (including ignorance as to the form of chromium in yeast), these reports cannot be used as emphasized by Mertz [116]. To guarantee proper experimental design and control, only placebo-controlled, double blind, crossover studies of the effects of chromium supplementation should be used. Given these very basic, minimum requirements, well-performed studies

with human subjects are surprisingly few in number. (Discussion of studies with diabetic subjects will be discussed separately (vide infra))

In a most thorough review in 1998, Anderson identified 23 previous studies in which the effects of chromium supplementation of non-diabetic subjects on blood variables or results of glucose tolerance tests were examined and identified similar studies on adult-onset diabetic subjects [114]. As most of these studies date to 1995 or earlier, this review will be used as the starting point for discussion. Two of these studies on non-diabetic subjects used yeast as a source of chromium [120, 121] and reported conflicting results; another study utilized a double blind format but used poorly defined "GTF-chromium" [122]. These studies will not be considered further. Of the remaining 20 studies [72, 123–141], amazingly only four studies meet the criteria outlined above. In addition, one study in the diabetes section of the review had a non-diabetic control group which also meets the criteria [142].

In 1968 Sherman et al. reported the effects of supplementing four adult males for 16 weeks with 150 μg Cr/day as $CrCl_3$ [142]. No effects were observed on fasting glucose or on glucose levels in oral glucose tolerance tests (0–180 minutes). Thomas and Gropper in 1997 observed no effects on blood total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides or on 90-minute blood insulin or glucose levels after an oral glucose load after supplementing 14 adults for 8 weeks with 200 μg Cr/day as CrNic [139]. In the double-blind, crossover study with the largest participant pool [131], Anderson and coworkers supplemented 76 adults for 12 weeks with 200 μg Cr/day as $CrCl_3$. When the total pool or all males or all females were considered, no changes were observed in fasting glucose, insulin, total cholesterol, HDL, LDL, VLDL, or triglyceride levels or on 90-minute glucose or insulin concentrations after an oral glucose challenge. However, significant effects were observed in certain sub-populations. Subjects (13 males and 7 females) with high glucose concentrations 90 minutes after a glucose challenge ($\geq$100 mg/dL) had lower fasting glucose concentrations and lower 90-minute glucose concentrations after supplementation. Subjects (13 males and 8 females) with 90-minute glucose levels lower than fasting glucose levels had increased levels of fasting glucose after supplementation. In a similar study in 1991, Anderson and coworkers found that eight control subjects supplemented for 4 weeks with 200 μg Cr/day as $CrCl_3$ had no observed changes in glucose or insulin levels during glucose tolerance tests (0–240 minutes) [140]. Nine subjects considered "hyperglycemic" (90-minute glucose levels between 100 and 200 mg/dL) had lower 60-minute glucose and insulin concentrations while the concentrations were similar at 0, 30, 90, 120, 180, and 240 minutes. All subjects in this study also consumed a "low-Cr" diet, although the significance of this is hard to evaluate as a control group on a self-selected or other diet was not included in the study. Finally, Evans and coworkers have examined 28 adults for 6 weeks supplemented with 200 μg Cr/day as $Cr(pic)_3$ [72]. Triglyceride and HDL cholesterol were unaffected by Cr supplementation. Total and LDL cholesterol levels were reported to be lower. However, the statistical analysis for this study was performed differently in this study than the others. Each subject was allowed to serve as his or her own control, and paired *t* values were calculated for each person. If just the average initial and final values of the total and LDL cholesterol levels and the associated SEM's are compared, the effects are not statistically significant at $P < 0.05$. Also, it is of note that in this study the initial total

and LDL levels differed significantly between the subjects started on the supplement and the group started on the placebo, with those on the placebo having lower initial values.

Another well-performed study of interest was performed using hypoglycemic subjects by Anderson, et al. [143] Eight female subjects were given 200 μg Cr/day as $CrCl_3$ for 12 weeks. No change was observed in total cholesterol, HDL, LDL, VLDL, or triglyceride levels. The area of glucose below the fasting glucose level in glucose tolerance tests was lowered after 6 weeks of supplementation but was not significantly changed after 12 weeks.

The consensus of these studies is that Cr supplementation of healthy adults has no effect on glucose, insulin, or blood lipids, while it is possible that subjects with marginally abnormal blood glucose levels after a glucose challenge may be affected beneficially by supplementation.

Perhaps the strongest evidence for an essential role of chromium in humans arises from studies of patients on total parenteral nutrition (TPN) [115, 144]. Patients on TPN have developed impaired glucose utilization [145] or glucose intolerance and neuropathy or encephalopathy [146–148]. The symptoms were reversed by chromium infusion and not other treatments. While limited to five individual cases, these studies provide evidence of clinical symptoms associated with Cr deficiency that can be reversed by supplementation. Another patient on TPN who developed symptoms of adult-onset diabetes and hyperlipidemia but died had low tissue Cr levels [149]. Curiously, the development of symptoms which were reversible by Cr supplementation does not correlate with serum Cr levels [115], indicating that serum Cr levels are not an indicator of Cr deficiency. These incidences have recently been reviewed [115, 150]. Additionally, these incidences of diagnosed potential Cr deficiency have been questioned recently as they lack consistent relationships between the Cr in the TPN, time on TPN before symptoms, serum Cr levels, and symptoms [151]. Hence, no known indicator of Cr deficiency exists other than reversal of symptoms upon Cr supplementation [115]; a marker for Cr status in tissues is desperately needed.

Additional evidence for a potential role for Cr comes from studies of Cr absorption as a function of chromium intake. Absorption varies inversely with intake as low intakes (~15 μg/day) lead to high rates of absorption (~2%) while high intake (~35 μg/day) reduces absorption to ~0.4% [152]. Work from the same research group indicates that absorption of Cr may not be inversely proportional to intake in rats [153].

A role for Cr in the body is also suggested by an association between insulin action and increased urinary loss, although other explanations may be possible. In human euglycemic hyperinsulinemic clamp studies, Morris and coworkers have shown that increases in blood insulin concentrations following an oral glucose load result in significant decreases in plasma chromium levels; a subsequent infusion of insulin led to further chromium losses [154]. Within one-and-a-half hours after the increases of blood insulin concentrations, blood chromium levels started to recover. Patients also showed increased urinary chromium losses during the course of the experiments, with the amount of chromium lost roughly corresponding to the amount of chromium estimated to be lost from the intravascular space [154]. Studies with human and rat tissue have demonstrated that chromium binding by insulin-dependent tissues is significantly enhanced by glucose, suggesting that chromium may translocate from the blood compartment to insulin-sensitive tissues [155]. (However, these studies used $CrCl_3$ as the chromium source; the

isolated tissue might lack a chromium transport agent such as transferrin in the blood (vide infra), making interpretation difficult.) Numerous studies have demonstrated that chromium is released in urine within 90 minutes of a dietary stress such as high sugar intake [156–161]. As glucose tolerance as a result of repeated application of carbohydrate stress decreases, the mobilization of chromium and resulting chromium loss have been shown to decrease [158].

The National Research Council (NRC) of the Food and Nutrition Board established the first Estimated Safe and Adequate Dietary Intake (ESADDI) for chromium in 1980 at 50–200 μg/day for adults [162]. There were insufficient data for developing a Recommended Daily Allowance (RDA). The ESADDI was left unchanged by the Council in 1989 [163]. ESADDI were also established for infants (10–40 μg/day), children (6–12 months of age; 20–40 μg/day), young children (20–80 μg/day), and adolescents (30–120 μg/day). The bases for the current ESADDI's has been reviewed by Hunt and Stoecker [164]. Based on these numbers, as much as 90% of American diets [152] and half of those of developed nations [165] reportedly may not consume the ESADDI for Cr. Levels of consumption for adults below 50 μg/day have been found on numerous occasions [56, 165–169], although equilibrium (positive net Cr balance) may be achieved at these levels [168, 169]. Based on these reports, the NRC in 2001 established a new guideline for Cr intake. The daily adequate intake (AI, a new term replacing ESSADI) was 35 μg/day for an adult male and 25 μg/day for an adult female [170]. The NRC recommendations are the focus of Chapter 2. This number sets an important guideline for assessing human studies of Cr supplementation. Studies using 200 μg Cr/day or greater should be considered pharmacologically relevant, rather than nutritionally relevant.

On the other extreme, limited data exists as to the level of dietary Cr(III) at which the ion becomes unsafe for humans [115, 171, 172]. The low level of absorption of dietary Cr(III) combined with the very high cell concentrations required before known toxic effects are manifested makes it unlikely that increasing dietary Cr levels can lead to health problems. Such considerations should not ignore the possibility that supplementation of the diet with synthetic chromium complexes such as $Cr(pic)_3$ could lead to adverse effects (vide supra). This will be a focus of Chapters 9–12.

**Other animals**

The effects of chromium supplementation of the diet have been examined with numerous animals including cows, pigs, sheep, chickens, horses, rabbits, and fish. Studies through early 1997 have been reviewed by the NRC [173]. Most studies have used $Cr(pic)_3$ or $CrCl_3$ as the chromium source although a few reports utilized chromium nicotinate or Cr-enriched yeast. The Committee on Animal Nutrition of the National Research Council noted that information was highly contradictory or too sparse to make recommendations. Studies on growing-finishing swine serve as an example. $Cr(pic)_3$ supplementation of the diet at levels of 200–500 μg Cr/kg resulted in statistically significant improvement in growth in 11 of 31 studies; feed efficiency improved in 8 of 31 studies [173]. (For significant improvement, $P$ values $\leq 0.10$ were used for these statistics rather than the more usual and rigorous requirement of $P \leq 0.05$.) Until a method for determining the Cr status of animals is available, interpretation of such inconsistent results is difficult at best. The results of studies since the NRC report are reviewed in Chapter 5.

## LOW-MOLECULAR-WEIGHT CHROMIUM-BINDING SUBSTANCE (LMWCr)

Insulin dose response studies using rat adipocytes have suggested a potential intrinsic biological function for the Cr-containing biomolecule, LMWCr. Isolated rat adipocytes in the presence of chromodulin and insulin display an increased ability to metabolize glucose to produce carbon dioxide or total lipids; this increase occurs without a change in the insulin concentration required for half maximal stimulation [20, 172, 174]. This lack of change in half-maximal insulin concentration suggests a potential role for LMWCr inside the insulin-sensitive cells after insulin binds externally to the insulin receptor [41]. The stimulation of glucose metabolism by LMWCr is proportional to the chromium content of the oligopeptide 175].

Low-molecular-weight chromium-binding substance is a naturally occurring oligopeptide composed of glycine, cysteine, aspartate, and glutamate with the carboxylates comprising more than half of the total amino acid residues [176, 177]. Despite its small size (approximately 1500 molecular weight), the molecule tightly binds four equivalents of chromic ions. By 1995 the oligopeptide had been isolated and purified from the liver of rabbit [176], and partially purified from the livers of dog [178] and mouse [177]. A related chromium-containing oligopeptide from bovine colostrum (M-LMWCr) is comprised of the same amino acids but in distinctly different ratios and also stimulates insulin-dependent glucose metabolism in rat adipocytes [172, 174]. Whether the oligopeptide is present in other forms of milk is unknown. The significance of these differences between the liver and the colostrum oligopeptides is essentially unexplored. The oligopeptide is isolated as the holo-oligopeptide (so that it may be followed in the purification schemes by its chromium content), which means that an in vivo chromium-loading step is required [176, 177]. This observation has resulted in the suggestion that LMWCr may play a role in chromium detoxification; however, injection of chromic ions or chromate into mice does not stimulate the production of LMWCr [177]. Thus, while LMWCr does carry chromium into the urine after intake of large dosages of Cr(III) or Cr(VI) [178], the suggested detoxification role of LMWCr is unlikely to be its primary function. Chromium in LMWCr in the lungs (of rats exposed to C-containing aerosol) has been proposed to be in equilibrium with Cr in the rest of the body; the long half-life of chromium in the lungs was proposed to be the result of low chromodulin levels or a slow rate of synthesis of the oligopeptide [177].

A proposed role for LMWCr in insulin signaling is reviewed in Chapter 7. Intravenous treatment of rats with 20 μg of chromium as LMWCr per day for 12 weeks has recently been shown to have little, if any, effect on rats [179]. Thus, in terms of its use as a dietary supplement or therapeutic compound, LMWCr may be recognized and readily excreted. Injection of chromodulin into rabbits has been shown to lead to rapid excretion of chromium, especially compared to use of other forms of chromium; this is reflected in the mean tubular reabsorption rate for chromodulin of 23.5% in contrast to rates of 85.7 and 92.5% for chromate and chromium chloride, respectively [178]. This is probably also responsible for the extremely high $LD_{50}$ for chromodulin injected into mice of 135 mg/kg body mass [180].

### Synthetic models

The recent research on chromodulin has inspired synthetic efforts to prepare new chromium–carboxylate assemblies. Known assemblies with nuclearity greater than two (but less than eight) possess four types of cores: symmetric [181] and unsymmetric [182] $Cr_3O$, $Cr_3(OH)_2$ [183], and $Cr_4O_2$ [184–186]. Numerous examples of the type containing the symmetric $Cr_3O$ core have been well characterized, and interest in these complexes date back to the late nineteenth century [181]. The physical properties of the $Cr_3O$ and related "basic carboxylate" complexes of other metals have been extensively reviewed [181]. The other cores have only been prepared during the last decade using the symmetric trinuclear complexes as starting materials.

## OTHER POTENTIAL CHROMIUM-CONTAINING BIOMOLECULES

In 1959 Wacker and Vallee reported a ribonucleoprotein from liver; the material contained 0.1% chromium in addition to a few other metals [187, 188]. Chromium could not be removed by the addition of chelating agents. The purity of the material is unfortunately impossible to gauge from the report. RNA from a number of other sources was then examined; all RNA samples contained appreciable quantities of chromium, suggesting chromium accumulates in or is specifically associated with RNA [187, 188]. These observations are worthy of re-examination. Does $Cr^{3+}$ just accumulate with RNA because of a propensity for chelating phosphate-type ligands or is there a specific role for chromium? This possible binding of chromium to nucleic acids could also potentially be related to possible toxic aspects of the metal (see Chapters 9–12).

Okada and coworkers have reported that $Cr^{3+}$ stimulated RNA synthesis in vitro [189], but the relevance of this study is uncertain. These workers have also found that rats injected with $CrCl_3$ and partially hepatectomized 24 hours later had increased hepatic nucleolar RNA synthesis accompanied by the production of a nucleolar Cr-containing protein of 70 kDa [190, 191]. Whether this is biologically significant or an artifact requires additional study. This group has also reported that a chromatin-Cr complex showed enhanced template activity for in vitro RNA synthesis [192,193].

## DIABETES AND CHROMIUM/CHROMIUM TRANSPORT

### Adult-onset diabetes and chromium

The potential relationship between chromium excretion and insulin signaling raises questions about potential associations between chromium action and diabetes. Over 120 million people across the planet have been estimated to have diabetes mellitus, with approximately 16 million of these in the United States. Between 90 and 95% of the US cases are of type 2 diabetes (also called adult-onset or non-insulin dependent diabetes). Type 2 diabetes is the form responsible for the rapid increase in diabetes cases over the last few decades, and the number of cases are increasing rapidly, especially in third-world countries. Obesity is a risk factor for adult-onset diabetes, and the occurrence of the syndrome increases with age; both the average age and rate of obesity are increasing in the United States. African Americans, American Indians, Hispanics, and Pacific Islanders are especially susceptible to the disease. Unlike juvenile diabetes, which is an

autoimmune disorder, type 2 diabetes results from insulin resistance, the body produces and releases insulin normally; however, the insulin signal is not properly transmitted into cells. With time, however, the beta cells of the pancreas break down resulting in reductions in insulin production. The cause of the disease at a molecular level has only been elucidated in a tiny fraction of cases. Consequently, any mechanism by which insulin signaling could be stimulated has possible value in the treatment of the symptoms of type 2 diabetes.

Several studies attempted to test the existence of a link between type 2 diabetes and chromium. Studies examining serum and urine Cr concentrations of healthy and diabetic subjects (using analytical techniques developed after *c*. 1980 and reporting serum or urine Cr levels of healthy subjects less than approximately 0.5 μg/L) [118] have found Cr levels of diabetics that are distinctly different from those of healthy individuals. Morris and coworkers have reported that serum levels of type 2 diabetics are approximately one-third lower than those of healthy subjects while urine Cr concentrations are about twice as high [194–196]. In the first 2 years of the onset of diabetes, plasma Cr levels inversely correlated with plasma glucose concentrations, but this trend disappeared for patients with the disease for longer duration. Increased urinary output in type 2 diabetics would be consistent with studies with healthy individuals that indicate increased urinary Cr output and decreased serum Cr levels in response to increases in serum glucose or insulin [156–161]. It should be noted that Anderson and coworkers have shown in a double-blind crossover, placebo-controlled study that serum Cr levels reflect Cr intake but reported no effect by a glucose challenge [197]. However, they only used serum glucose values 90 minutes after glucose treatment; in studies with multiple time points from 0 to 180 minutes after glucose treatment, serum chromium decreases but is restored to near original concentrations by 90 minutes [154, 160, 161].

In his 1998 review article, Anderson [114] identified 16 studies of chromium supplementation of type 2 diabetic subjects [68, 120, 122, 126, 139, 141, 142, 198–206]. However, only six of these studies were placebo-controlled, double-blind crossover in design. The first in 1968 by Sherman and coworkers giving seven adult males 150 μg Cr/day as $CrCl_3$ for 16 weeks observed no effect on fasting glucose levels and no effects on glucose tolerance tests (0–180 minutes after glucose administration) [142]. Rabinowitz and coworkers in a larger study in 1983 gave 43 adult men the same amount of $CrCl_3$ for the same length of time [198]. No effects were observed on fasting blood levels of cholesterol, triglycerides, or glucose or observed in glucose levels 2 hours after subjects consumed a meal. Similar results were obtained in 1983 by Uusitupa and coworkers working with ten adults that were supplemented for 6 weeks with 200 μg of Cr as $CrCl_3$ per day [199]. No effects were observed on total cholesterol, triglycerides, HDL, LDL, or VLDL cholesterol, or fasting glucose or insulin levels. No effects were observed either in glucose levels 1 or 2 hours or in insulin levels 2 hours after an oral glucose challenge; however, insulin levels 1 hour after the challenge were significantly lower. No effects on total cholesterol, HDL or LDL cholesterol, triglycerides, or glucose and insulin levels 90 minutes after a glucose challenge were observed by Thomas and Gropper in 1997 [139]. These researchers used a pool of five adults which were given 200 μg of Cr as CrNic per day for 8 weeks. In 1994, Lee and Reasner [200] examined 28 adult diabetic subjects given 200 μg/day $Cr(pic)_3$ for 8 weeks. (It is possible that the authors meant to indicate that subjects were given 200 μg/day Cr, not $Cr(pic)_3$,

but this is not how the experimental description reads.) No effect was observed on HDL or LDL cholesterol levels or fasting glucose levels. Triglyceride levels were lower significantly. Contrasting results were obtained by Gary Evans in 1989 [68]. Using 11 adults given 200 μg Cr as $Cr(pic)_3$ for 6 weeks, Evans noted significantly lower fasting glucose, total cholesterol, and LDL cholesterol concentrations. Evans used paired *t*-tests to determine the significance of the difference of the means (as previously described). This gives results such as initial and final concentrations of LDL cholesterol (mean ± SE) of $148 \pm 12$ vs. $140 \pm 11$ and of $142 \pm 11$ vs. $134 \pm 12$ to be significant ($P \leq 0.05$). The reportedly lower glucose and cholesterol concentrations disappear when standard statistical treatment is used.

While type 2 diabetes is associated with abnormally low serum Cr and high urine Cr levels possibly related to elevated serum glucose and insulin levels, the consensus of these studies appears to be that Cr supplementation for 6–16 weeks has no effect on diabetic subjects. This begs the question of the significance of the increased Cr movement. Do diabetics improperly transport Cr? Is Cr movement the result of some other phenomenon such as altered iron metabolism? Do diabetics slowly deplete Cr stores? However, since 1995, many studies examining the effects of Cr on type 2 diabetics have been reported. These studies are the focus of Chapter 8.

**Other conditions resulting in increased urinary Cr loss**

Three other conditions reported to result in increased urinary Cr loss are trauma, exercise, and pregnancy. In the case of trauma [207, 208], urinary Cr excretion is very high but appears to decrease rapidly. Effects of Cr intake are difficult to analyze as intake varies dramatically depending on patient treatment. The appropriate level of Cr in TPN solutions has been an issue of debate, but that debate is beyond the scope of this review. Acute exercise-induced changes associated with increased glucose utilization have been found to result in increased urinary chromium excretion in several human studies [71, 157, 209–211], but not all [69, 70]. Clarkson has determined that insufficient evidence of any beneficial effects existed to recommend chromium supplementation for athletes [212].

Unfortunately, data on any potential relationship between Cr and pregnancy and especially gestational diabetes are sparse, especially after 1980 and the use of reliable analytical techniques to determine tissue and fluid Cr concentrations. Patients in the first half of pregnancy have been reported to have higher Cr excretion [213]. Patients in the second half of pregnancy had urinary Cr levels 30% higher than controls, but the difference was not significant. The concentration of Cr in hair from women with gestational diabetes appears to be lower than that of controls [213]. Jovanovic has presented results of chromium supplementation of women with gestational diabetes [214]. Chromium was reported to lower glucose and insulin levels compared to controls. If the results of this study are reproduced by additional studies, this could have significant implications for treatment of this condition.

**Chromium transport**

If chromium is an essential element, then a specific transport mechanism should exist for the element. However, the existence of such a mechanism is not proof that the element is essential, as toxic metals are also recognized, transported, and excreted.

The mechanisms of absorption and transport of chromic ions are still uncertain. Little is known of the fate of $Cr^{3+}$ that is taken orally. As a start, essentially no data exists on the forms of Cr(III) in food as a result of its very low concentration. Presumably, some small percentage of chromium in mammalian tissues is LMWCr. However, the fate of this or other forms of dietary chromium in the digestive tract (exposure to proteases and other hydrolases, highly acidic pH of the stomach, etc.) is also unknown. As described earlier, only a small percentage (<2%) of dietary Cr is absorbed, while the remainder is excreted in the feces. Chromium supplementation of the diet results in an increase in urinary chromium loss, and most absorbed chromium is rapidly excreted [215]. Dowling and coworkers have examined the absorption of $Cr^{3+}$ from an intestinal perfusate with added Cr as $CrCl_3$ [216]. Cr absorption was found to be a nonsaturable process, leading to the conclusion that the $Cr^{3+}$ was "absorbed by the nonmediated process of passive diffusion in the small intestine of rats fed a Cr-adequate diet." This study was methodologically superior to previous studies which have yielded conflicting results [11, 217, 218]. Yet, the fate of dietary Cr(III) organic complexes could be different than that of the form(s) of chromium formed in the perfusate. For example, the presence of added amino acids, phyate (high levels), and oxalate in the diet reportedly alter Cr uptake [219, 220], as does ascorbic acid [221]. Low levels of phyate appear to have no effect on absorption [222]. Absorption of dietary Cr may be more complicated than simple passive absorption. The perfusate studies also would appear to contradict the inverse relationship between dietary chromium intake and degree of absorption observed in human studies [152]; the authors of the human study have also examined absorption of chromium by rats [153]. They proposed that Cr homeostasis was maintained at the level of excretion, not absorption, and suggested Cr uptake by rats may be different from that in humans.

The fate of chromium in the bloodstream is somewhat better elucidated. In vivo administration of chromic ions to mammals by injection results in the appearance of chromic ions in the iron-transport protein transferrin. In 1964, Hopkins and Schwarz established $^{51}CrCl_3$ given by stomach tube to rats resulted in $\geq$99% of the chromium in blood being associated with non-cellular components [223]. Ninety percent of the Cr in blood serum was associated with the $\beta$-globulin fractions; 80% immunoprecipitated with transferrin [223]. In vitro studies of the addition of chromium sources to blood or blood plasma also result in the loading of transferrin with Cr(III), although under these conditions albumin and some degradation products also bind chromium [224, 225]. In vitro studies suggest transferrin may be important for transport from the intestines [226]. Transferrin is an 80,000 Da blood serum protein that tightly binds two equivalents of ferric iron at neutral and slightly basic pH's. The protein exhibits amazing selectively for Fe(III) in a biological environment because the metal sites are adapted to bind ions with large charge-to-size ratios. In humans, transferrin is maintained only approximately 30% loaded with iron on average and consequently has been proposed to potentially carry other metal ions [227]. The similar charge and ionic radii of chromic ions to ferric ions suggest that chromic ions should bind relatively tightly to the protein. In vitro studies of the addition of chromic ions to isolated transferrin reveal that Cr(III) readily binds to the two metal-binding sites, resulting in intense changes in the protein's ultraviolet spectrum [228–234]. The two chromium-binding sites can be distinguished by EPR, and only chromic ions at one site can be displaced by iron at near neutral pH [229, 231]. Below pH 6, only one site binds chromium [232].

As a result of the in vivo and in vitro studies, it has been assumed reasonably that transferrin was involved in chromium transport, although by 1995 transport had not conclusively demonstrated in vivo. Could increased loading of transferrin with iron prevent adequate chromium binding and transfer by transferrin, resulting in insulin resistance and diabetes? The hemochromatotic diabetic condition is certainly exacerbated by reduced chromium retention [235], as observed in adult-onset diabetic patients. Recent advances on elucidating the mechanism transport of Cr are reviewed in Chapter 6, while association between the transport of Cr and potential roles in insulin signaling are described in Chapter 7.

Only minor amounts of chromium are lost through the bile (for example, [236, 237]). In bile, $Cr^{3+}$ occurs as part of a low-molecular-weight organic complex [238]; the molecular weight of this species was not determined. The authors postulated that this complex might be involved in passage of chromium from the liver to the bile.

Exactly how chromium is handled by the kidneys is difficult to determine from the literature. Some useful tracer studies with $^{51}Cr$ have been reported (for example, see [239–241]); yet, much of the literature looking at absolute Cr levels dates before 1980 and, thus, suffers from analytical problems. The tracer studies appear to be influenced by the form of chromium used and perhaps by the species of animal utilized. Similar problems with chromium form are associated with studies examining Cr distribution in mammals. Differences may exist between studies that give Cr orally or intravenously as different Cr(III) species may be introduced into the blood. Similarly, tracer studies may not accurately reflect the fate of dietary chromium; for example, inorganic chromium added to a diet may not bind to the same chelates and other ligands binding to the chromium naturally in the diet. Yet, some consensus does appear. $^{51}Cr$ in tracer studies accumulates in the bone, kidney, spleen, and liver (for example, [11, 107, 236, 241–245]). A three-compartment model has been proposed to examine the kinetics of chromium tissue exchange and distribution for studies with rats and humans[11, 242, 243]. Plasma chromium is in equilibrium with the three pools: a small pool with rapid exchange ($T_{1/2} < 1$ day), a medium pool with a medium rate of exchange (days), and a large, slowly exchanging pool (months). Jain and coworkers have suggested from these studies that chromium has specific transporters that regulate its movement [107]. Aging appears to affect Cr distribution and transport [245].

## CONCLUSION

By 1995, the status of Cr as an essential trace was starting to become controversial, while the element was almost uniformly asserted to be essential for the previous four decades. The initial work on GTF and “GTF” had been found to have significant methodological shortfalls and certainly by current standards must be neglected in terms of arguments for the essentialness of Cr. The author wishes to stress his believe that the use of the term GTF contains so much historical baggage that it should be abandoned. These studies have led, however, to the development of the chromium nutritional supplement industry, whose major product is chromium picolinate. Only the beginning of the story of this supplement can be told in this review. In fact, subsequent chapters in this book describing the use of this supplement will likely not be the final word on the issues of its efficacy and toxicity.

Much is still left to be determined about the transport and distribution of Cr, although some progress has been made in this area since 1995. If Cr is essential, it should have a specific transport and distribution system in the body. However, the elucidation of such a system would not prove that Cr is essential as such systems also exist for certain toxins.

The evidence for an essential role of Cr to this point can be summarized as follows:

1. Rats fed a "low-Cr", sucrose-based diet may have increased insulin areas in glucose tolerance tests, suggesting the development of tissue insulin resistance. However, the development of Cr deficiency in any rat model is now known to be unlikely.
2. Apparent Cr deficiency has been developed by a limited number of patients on TPN, resulting in symptoms suggestive of the symptoms of type 2 diabetes. The symptoms of this condition were reversed by chromium supplementation. The interpretation of these studies is currently a matter of debate, and TPN solutions are now supplemented with Cr.
3. Cr absorption is inversely proportional to intake. Potential differences reported for the mechanism of absorption between humans and rats suggest that the results of this study need to be reproduced [246].
4. Increases in serum glucose result in increases in urinary chromium excretion. Conditions which alter glucose metabolism (including type 2 diabetes and exercise) alter urinary Cr output. These results could suggest a relationship between chromium and proper glucose metabolism. The relationship between insulin action and chromium transport, distribution, and excretion is currently an important area of research.

Fortunately, generating chromium deficiency is difficult, although this suggests that dietary chromium supplements are of limited (if any) value for healthy individuals; chromium supplementation has not been found to have any beneficial effects on healthy humans. No medical conditions can unequivocally be related to Cr deficiency. Conditions such as type 2 diabetes with long-term increases in urinary chromium loss may in theory generate marginal chromium deficiency which potentially could be alleviated by supplementation; however, this has yet to be demonstrated convincingly. In fact, if chromium supplements are found to be effective in alleviating the symptoms of type 2 diabetes and other conditions, supplemental Cr may have a completely different mode of action than restoring Cr nutritional balance. Research on the effects of Cr on the symptoms of type 2 diabetes is the hottest current area of chromium research.

Yet, if Cr is an essential trace element, Cr presumably must bind to some biomolecule. Candidates proposed by 1995 have either been dismissed ("GTF" or $Cr(pic)_3$) or remain highly controversial (LMWCr). Other candidates have since been suggested but are likewise controversial. If a naturally occurring Cr-containing biomolecule could unequivocally be shown to perform a function in vivo (assuming such a molecule exists), this would be a major advance in the field.

Issues of potential toxicity of Cr(III), especially from supplements providing doses in orders of magnitude larger than the AI, were beginning to be explored in 1995. This has been a particularly contentious area of research, particularly given the potential financial implications.

Thus, after the first 40 years of study, little has been resolved; and as will be revealed in the remainder of the book, few significant advances in understanding have resulted in the last decade. In fact, even the basic question of whether Cr is an essential element is debated more now than 10 years ago. One interesting question is whether Cr is an essential element but is required in such low amounts that generating deficiency is next to impossible. Such a situation has been proposed (most controversially) for other elements such as arsenic and silicon. Would traditional nutritional studies be able to discern this situation?

Hopefully, the following chapters will spark new ideas and research into the nutritional status and biochemistry, leading to the resolution of these issues.

## ACKNOWLEDGMENTS

Work on Cr(III) in the laboratory of John B. Vincent has been funded by the NRICGP/USDA, NIH, American Diabetes Association, American Heart Association, and American Chemical Society/PRF. John B. Vincentis also the inventor or co-inventor of five patents on the use of chromium-containing oligopeptides or synthetic Cr(III) compounds as nutritional supplements or therapeutic agents.

## REFERENCES

[1] Vincent, J. B. (2001) The bioinorganic chemistry of chromium(III). *Polyhedron 20*, 1–26.
[2] Mertz, W., and Schwarz, K. (1955) Impaired intravenous glucose tolerance as an early sign of dietary necrotic liver degeneration. *Arch. Biochem. Biophys. 58*, 504–506.
[3] Schwarz, K., and Mertz, W. (1957) A glucose tolerance factor and its differentiation from factor 3. *Arch. Biochem. Biophys. 72*, 515–518.
[4] Schwarz, K., and Mertz, W. (1959) *Arch. Biochem. Biophys. 85*, 292–295.
[5] Mertz, W., Roginski, E. E., and Schwarz, K. (1961) Effect of trivalent chromium complexes on glucose uptake by epididymal fat tissue of rats. *J. Biol. Chem. 236*, 318–322.
[6] Mertz, W., and Schwarz, K. (1962) An effect of liver extracts on glucose tolerance in rats. *Am. J. Physiol. 203*, 53–56.
[7] Mertz, W., and Schwarz, K. (1959) Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. *Am. J. Physiol. 196*, 614–618.
[8] Mertz, W., Roginski, E., and Schroeder, H. A. (1965) Some aspects of glucose metabolism of chromium-deficent rats raised in a strictly controlled enviroment. *J. Nutr. 86*, 107–112.
[9] Roginski, E. E., and Mertz, W. (1967) An eye lesion in rats fed low chromium diets. *J. Nutr. 93*, 249–251.
[10] Mertz, W., and Roginski, E. E. (1963) The effect of trivalent chromium on galactose entry in rat epididymal fat tissue. *J. Biol. Chem. 238*, 868–872.
[11] Mertz, W., Roginski, E. E., and Reba, R. C. (1965) Biological activity and fate of trace quantities of intravenous chromium(III) in the rat. *Am. J. Physiol. 209*, 489–494.
[12] Mertz, W. (1975) Effects and metabolism of glucose tolerance factor. *Nutr. Rev. 33*, 129–135.
[13] Mertz, W., Toepfer, E. W., Roginski, E. E., and Polansky, M. M. (1974) Present knowledge of the role of chromium. *Fed. Proc. 33*, 2275–2280.
[14] Christian, G. D., Knoblock, C. E., C.Purdy, W., and Mertz, W. (1963) A polarographic study of chromium-insulin–mitochondrial interaction. *Biochim. Biophys. Acta 66*, 420–423.

[15] Cambell, W. J., and Mertz, W. (1963) Interaction of insulin and chromium(III) on mitochondrial swelling. *Am. J. Physiol. 204*, 1028–1030.
[16] Evans, G. W., Roginski, E. E., and Mertz, W. (1973) Interaction of the glucose tolerance factor (GTF) with insulin. *Biochem. Biophys. Res. Commun. 50*, 718–722.
[17] Votava, H. J., Hahn, C. J., and Evans, G. W. (1973) Isolation and partial characterization of a 51Cr complex from brewers' yeast. *Biochem. Biophys. Res. Commun. 55*, 312–319.
[18] Toepfer, E. W., Mertz, W., Polansky, M. M., Roginski, E. E., and Wolf, W. R. (1977) Preparation of chromium-containing material of glucose tolerance factor activity from brewer's yeast extracts and by synthesis. *J. Agric. Food Chem. 25*, 162–162.
[19] Anderson, R. A., and Mertz, W. (1977) Glucose tolerance factor: an essential dietary agent. *Trends Biochem. Sci. 2*, 277–279.
[20] Kumpulainen, J., Koivistoinen, P., and Lahtinen, S. (1978) Isolation, purification, and partial chemical characterization of chromium(III) fractions existing in brewer's yeast and Sabouraud's liquid medium. *Bioinorg. Chem. 8*, 419–429.
[21] Gonzalez-Vergara, E., Hegenauer, J., and Saltman, P. (1982) Biological complexes of chromium: A second look at glucose tolerance factor. *Fed. Proc. 41*, 286.
[22] Held, D. D., Gonzalez-Vergara, E., and Goff, H. M. (1984) Isolation of a non-chromium insulin-enhancing factor from brewer's yeast. *Fed. Proc. 43*, 472.
[23] Haylock, S. J., Buckley, P. D., and Blackwell, L. F. (1983) Separation of biologically active chromium-containing complexes from yeast extracts and other sources of glucose tolerance factor (GTF) activity. *J. Inorg. Biochem. 18*, 195–211.
[24] Mirsky, N., Weiss, A., and Dori, Z. (1980) Chromium in biological systems, I. Some observations on glucose tolerance factor in yeast. *J. Inorg. Biochem. 13*, 11–21.
[25] Mirsky, N., Weiss, A., and Dori, Z. (1981) The effect of glucose tolerance factor on glucose uptake by yeast cells. *J. Inorg. Biochem. 15*, 275–279.
[26] Holdsworth, E. S., and Appleby, G. (1984) Assays of glucose tolerance factor and its mode of action, studied with brewer's yeast. *J. Inorg. Biochem. 21*, 31–44.
[27] Mirsky, N. (1993) Glucose tolerance factor reduces blood glucose and free fatty acids levels in diabetic rats. *J. Inorg. Biochem. 49*, 123–128.
[28] Mirsky, N., and Berdicevsky, I. (1994) Effects of insulin and glucose tolerance factor on glucose uptake by yeast cells. *Biol. Signals 3*, 271–277.
[29] Berdicevsky, I., and Mirsky, N. (1994) Effects of insulin and glucose tolerance factor (GTF) on growth of Saccharomyces cerevisiae. *Mycoses 37*, 405–410.
[30] Muller, G., Rouveyre, N., Crecelius, A., and Bandlow, W. (1998) Insulin signaling in the yeast Saccharomyces cerevisiae. 1. Stimulation of glucose metabolism and Snf1 kinase by human insulin. *Biochemistry 37*, 8683–8695.
[31] Muller, G., Rouveyre, N., Upshon, C., Gross, E., and Bandlow, W. (1998) Insulin signaling in the yeast Saccharomyces cerevisiae. 2. Interaction of human insulin with a putative binding protein. *Biochemistry 37*, 8696–8704.
[32] Muller, G., Rouveyre, N., Upshon, C., and Bandlow, W. (1998) Insulin signaling in the yeast Saccharomyces cerevisiae. 3. Induction of protein phosphorylation by human insulin. *Biochemistry 37*, 8705–8713.
[33] Haylock, S. J., Buckley, P. D., and Blackwell, L. F. (1983) The relationship of chromium to the glucose tolerance factor. II. *J. Inorg. Biochem. 19*, 105–117.
[34] Davies, D. M., Holdsworth, E. S., and Sherriff, J. L. (1985) The isolation of glucose tolerance factors from brewer's yeast and their relationship to chromium. *Biochem. Med. 33*, 297–311.
[35] Holdsworth, E. S., and Neville, E. (1988) Extracts of brewer's yeast contain GABA which enhances activation of glycogen synthetase by insulin in isolated rat hepatocytes. *Biochem. Int. 17*, 1107–1116.

[36] Holdsworth, E. S., and Neville, E. (1990) Effects of extracts of high- and low-chromium brewer's yeast on metabolism of glucose by hepatocytes from rats fed on high- or low-Cr diets. *Br. J. Nutr. 63*, 623–630.
[37] Holdsworth, E. S., Kaufman, D. V., and Neville, E. (1991) A fraction derived from brewer's yeast inhibits cholesterol synthesis by rat liver preparations in vitro. *Br. J. Nutr. 65*, 285–299.
[38] Hwang, D. L., Lev-Ran, A., Papoian, T., and Beech, W. K. (1987) Insulin-like activity of chromium-binding fractions from brewer's yeast. *J. Inorg. Biochem. 30*, 219–225.
[39] Simonoff, M., Shapcott, D., Alameddine, S., Sutter-Dub, M. T., and Simonoff, G. (1992) The isolation of glucose tolerance factors from Brewer's yeast and their relation to chromium. *Biol. Trace Elem. Res. 32*, 25–38.
[40] Anderson, R. A., Brantner, J. H., and Polansky, M. M. (1978) An improved assay for biologically active chromium. *J. Agric. Food Chem. 26*, 1219–1221.
[41] Vincent, J. B. (1994) Relationship between glucose tolerance factor and low-molecular-weight chromium-binding substance. *J. Nutr. 124*, 117–119.
[42] Mertz, W. (1994) Reply to the letter of Dr. Vincent. *J. Nutr. 124*, 119.
[43] Gutierrez, J., Mertz, W., Toepfer, E. W., Polansky, M. M., and Roginski, E. E. (1974) Saccharomyces carlsbergensis: microbiological assay for unidentified factor related to glucose tolerance. *J. Agric. Food Chem. 22*, 100–103.
[44] Sumrall, K. H., and Vincent, J. B. (1997) Is glucose tolerance factor an artifact produced by acid hydrolysis of low-molecular-weight chromium-binding substance? *Polyhedron 16*, 4171–4177.
[45] Woolliscroft, J., and Barbosa, J. (1977) Analysis of chromium induced carbohydrate intolerance in the rat. *J. Nutr. 107*, 1702–1706.
[46] Shepherd, P. R., Elwood, C., Buckley, P. D., and Blackwell, L. F. (1992) Glucose tolerance factor putentiation of insulin action in adipocytes from rats raised on a *Torula* yeast diet cannot be attributed to a deficiency of chromium. *Biol. Trace Elem. Res. 32*, 109–113.
[47] Gonzalez-Vergara, E., Gonzalez, B. C. D., Hegenauer, J., and Saltman, P. (1981) Chromium coordination compounds of pyridoxal and nicotinic acid: Synthesis, absorptionh and metabolism. *Israel J. Chem. 21*, 18.
[48] Gonzalez-Vergara, E., Hegenauer, J., Saltman, P., Sabat, M., and Ibers, J. A. (1982) Synthesis and structure of a trinuclear chromium(III)-nicotinic acid complex. *Inorg. Chim. Acta 66*, 115–118.
[49] Gerdom, L. E., and Goff, H. M. (1982) Ligation modes for nicotinic acid binding to the chromium(III) salen complex. *Inorg. Chem. 21*, 3847–3848.
[50] McArdle, J. V., de Laubenfels, E., Shorter, A. L., and Ammon, H. L. (1982) The synthesis and structure of bis(methylnicotinate)aquatrichlorochromium(III). *Polyhedron 1*, 471–474.
[51] Chang, J. C., Gerdom, L. E., Baenziger, N. C., and Goff, H. M. (1983) Synthesis and molecular structure determination of carboxyl bound nicotinic acid (niacin) complexes of chromium(III). *Inorg. Chem. 22*, 1739–1744.
[52] Cooper, J. A., Anderson, B. F., Buckley, P. D., and Blackwell, L. F. (1984) Structure and biological activity of nitrogen and oxygen coordinated nicotinic acid complexes of chromium. *Inorg. Chim. Acta 91*, 1–9.
[53] Green, C. A., Bianchini, R. J., and Legg, J. I. (1984) Characterization of a stable chromium(III)-nicotinic acid complex by deuteron NMR. *Inorg. Chem. 23*, 2713–2715.
[54] Cooper, J. A., Blackwell, L. F., and Buckley, P. D. (1984) Chromium(III) complexes and their relationship to the glucose tolerance factor. Part II. Structure and biological activity of amino acid complexes. *Inorg. Chim. Acta 92*, 23–31.
[55] Barrett, J., Kormoh, M. K., and O'Brien, P. (1985) Preparation and characterization of some Cr(III) chloro-complexes with nicotinic acid esters. *Inorg. Chim. Acta 107*, 269–274.

[56] Broderick, W. E., and Legg, J. I. (1985) Synthesis of the first stable nitrogen coordinated nicotinic acid chromium(III) complexes: cis- and trans-H[$Cr(mal)_2(nic\text{-}N)_2$]. *Inorg. Chem. 24*, 3724–3725.

[57] Vicens, M., Fiol, J. J., Terron, A., Moreno, V., Goodgame, D. M. L., and Sheppard, R. N. (1992) Some new chromium(III) complexes of nicotinic acid; a D NMR and EPR study. *Inorg. Chim. Acta 192*, 139–142.

[58] Barrett, J., O'Brien, P., and Jesus, J. P. D. (1985) Chromium(III) and the glucose tolerance factor. *Polyhedron 4*, 1–14.

[59] Green, C. A., Kione, N., Legg, J. I., and Willet, R. D. (1990) Synthesis and characterization of μ-carboxylato-*O*, O-μ-hydroxo chromium(III) complexes containing amino acids, acetate and nicotinate as bridging ligands. Crystal structure of $K_2$[Cr(nta)(OH)(acetate-*O*, O)Cr(nta)]·$4H_2O$. *Inorg. Chim. Acta 176*, 87–93.

[60] Kingry, K. F., Rowyer, A. C., and Vincent, J. B. (1998) Nuclear magnetic resonance studies of chromium(III) pyridinecarboxylate complexes. *J. Inorg. Biochem. 72*, 79–88.

[61] Broadhurst, C. L., Schmidt, W. F., III, J. B. R., Polansky, M. M., Gautschi, K., and Anderson, R. A. (1997) Characterization and structure by NMR and FTIR spectroscopy, and molecular modeling of chromium(III) picolinate and nicotinate complexes utilized for nutritional supplementation. *J. Inorg. Biochem. 66*, 119–130.

[62] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Gautschi, K. (1996) Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. *J. Trace Elem. Exp. Med. 9*, 11–25.

[63] Evans, G. W., and Pouchnik, D. J. (1993) Composition and biological activity of chromium-pyridine carboxylate complexes. *J. Inorg. Biochem. 49*, 177–187.

[64] Olin, K. L., Stearns, D. M., Armstrong, W. H., and Keen, C. L. (1994) Comparative retention/absorption of chromium ($^{51}Cr$) from Cr chloride, $^{51}Cr$ nicotinate and $^{51}Cr$ picolinate in a rat model. *Trace Elem. Electrocytes 11*, 182–186.

[65] Bradshaw, J. E., Grossie, D. A., Mullica, D. F., and Pennington, D. E. (1988) Preparations and characterizations of $\mu_3$-oxo-hexakis ($\mu_2$-carboxylatopyridine-*O,O*) triaquatrichromium(III) perchlorates. *Inorg. Chim. Acta 141*, 41–47.

[66] Evans, G. W., and Bowman, T. D. (1992) Chromium picolinate increases membrane fluidity and rate of insulin internalization. *J. Inorg. Biochem. 46*, 243–250.

[67] Hellerstein, M. K. (1998) Is chromium supplementation effective in managing type II diabetes? *Nutr. Rev. 56*, 302–306.

[68] Evans, G. W. (1989) The effect of chromium picolinate on insulin controlled parameters in humans. *Int. J. Biosci. Med. Res. 11*, 163–180.

[69] Clancy, S. P., Clarkson, P. M., Cheke, M. E. D., Nosaka, K., Freedson, P. S., Cunningham, J. J., and Valentine, B. (1994) Effects of chromium picolinate supplementaion on body composition, strength, and urinary chromium loss in football players. *Int. J. Sport Nutr. 4*, 142–153.

[70] Hallmark, M. A., Reynolds, T. H., DeSouza, C. A., Dotson, C. O., Anderson, R. A., and Rogers, M. A. (1996) Effects of chromium and resistive training on muscle strength and body composition. *Med. Sci. Sports Exerc. 28*, 139–144.

[71] Lukaski, H. C., Bolonchuk, W. W., Siders, W. A., and Milne, D. B. (1996) Chromium supplementation and resistance training: Effects on body composition, strength, and trace element status of men. *Am. J. Clin. Nutr. 63*, 954–965.

[72] Press, R. I., Geller, J., and Evans, G. W. (1990) The effect of chromium picolinate on serum cholesterol and apolipoprotein fractions in human subjects. *West. J. Med. 152*, 41–45.

[73] Evans, G. W., and Meyer, L. (1992) Chromium picolinate increases longevity. *Age 15*, 134.

[74] Lefavi, R. G. (1993) Response. *Int. J. Sport Nutr. 3*, 120–122.

[75] Lefavi, R. G., Anderson, R. A., Keith, R. E., Wilson, G. D., McMillan, J. L., and Stone, M. H. (1992) Efficacy of chromium supplementation in athletes: emphasis on anabolism. *Int. J. Sport Nutr. 2*, 111–122.

[76] Neilsen, F. H. (1996) Controversial chromium: Does the superstar mineral of the Montebanks receive appropriate attention from clinicians and nutritionists? *Nutr. Today 31*, 226–233.

[77] Stearns, D. M., Wise, Sr., J. P., Patierno, S. R., and Wetterhahn, K. E. (1995) Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. *FASEB J. 9*, 1643–1648.

[78] Stearns, D. M., Belbruno, J. J., and Wetterhahn, K. E. (1995) A prediction of chromium(III) accumulation in humans from chromium dietary supplements. *FASEB J. 9*, 1650–1657.

[79] Saraceno, A. J., and Block, B. P. (1963) Inorganic coordination polymers. IV. The attempted replacement of acetylacetonate ligands with ligands picolinate. *Inorg. Chem. 2*, 864–865.

[80] Stearns, D. M., and Armstrong, W. H. (1992) Mononuclear and binuclear chromium(III) picolinate complexes. *Inorg. Chem. 31*, 5178–5184.

[81] Bhattacharya, M., and De., G. S. (1981) Kinetics of substitution of aquo ligands from hexaaquochromium(III) ion by picolinic acid in water-ethanol medium. *Indian J. Chem. 20A*, 780.

[82] Borisova, N. N., Shabilalov, A. A., and Azizov, M. A. (1987) Chromium(III) coordination compounds with 2-pyridinecarboxylic acid. *Koord. Khim. 13*, 1050–1054.

[83] Campanella, L., Ciacchierini, E., Angelis, G. D., and Petrone, V. (1977) The reaction between chromium(III) and picolinic acid. *Ann. Chim. 67*, 385.

[84] Ley, H., and Ficken, K. (1917) Isomerie und lichtabsorption bei inneren komplexsalzen. (Uber innere komplexsalze. XV.) *Ber. 50*, 1123.

[85] Vinayakumar, C. K., Dey, G. R., Kishore, K., and Moorthy, P. N. (1996) Redox reactions of V(III) and Cr(III)picolinate complexes in aqueous solutions. *Radiat. Phys. Chem. 48*, 737–742.

[86] Dorsett, T. E., and Walton, R. A. (1976) Studies on Metal Carboxylates.10. Reactions of group-6 hexacarbonyls with pyridinecarboxylic acids - complexes of chromium-(III), molybdenum(III), and tungsten-(0), -(I), and -(IV). *J. Chem. Soc.*, 347–350.

[87] Gould, E. S., and Taube, H. (1964) Electron transfer through organic structural units. aromatic and heterocyclic carboxylates as bridging groups in oxidation-reduction reactions. *J. Am. Chem. Soc. 86*, 1318–1328.

[88] Yuen, G., Heaster, H., and Hoggard, P. E. (1983) Amine spectrochemical properties in tris(aminocarboxylate) complexes of chromium(III). *Inorg. Chim. Acta 73*, 231–234.

[89] Israily, N. (1966) Preparation des isomeres *cis* et *trans* des complexes du chrome et des aminomonoacids. Etude des spectres d'absorption infrarouge et visible. *C. R. Acad. Sci. Paris. 262*, 1426–1428.

[90] Chakov, N. E., Collins, R. A., and Vincent, J. B. (1999) A re-investigation the electronic spectra of chromium(III) picolinate complexes and high yield synthesis and characterization of $Cr_2(\mu\text{-}OH)_2(pic)_4.5H_2O$ (Hpic = picolinic acid). *Polyhedron 18*, 2891–2897.

[91] Parajn-Costa, B. S., Wagner, C. C., Baran, E. J., and Anorg, Z. (2003) Voltammetric and spectroscopic study of chromium(III)/picolinate complexes. *Z. Anorg. Allg. Chem. 629*.

[92] Anderson, R. A. (1986) Chromium metabolism and its role in disease processes in man. *Clin. Physiol. Biochem. 4*, 31–41.

[93] Striffler, J. S., Polansky, M. M., and Anderson, R. A. (1999) Overproduction of insulin in the chromium-deficient rat. *Metabolism 48*, 1063–1068.

[94] Schwarz, K. (1951) Production of dietary necrotic liver degeneration using American Torula yeast. *Proc. Soc. Exp. Biol. Med. 77*, 818–823.

[95] Schroeder, H. A., Vinton, W. H., Jr, and Balassa, J. J. (1963) Effects of chromium, cadmium and lead on the growth and survival of rats. *J. Nutr. 80*, 48–54.

[96] Schroeder, H. A. (1965) Diabetic-like serum glucose levels in chromium deficient rats. *Life Sciences 4*, 2057–2062.
[97] Schroeder, H. A. (1966) Chromium deficiency in rats: A syndrome simulating Diabetes mellitus with retarded growth. *J. Nutr. 88*, 439–445
[98] Schroeder, H. A. (1968) Serum cholesterol levels in rats fed thirteen trace elements. *J. Nutr. 94*, 475–480.
[99] Schroeder, H. A. (1969) Serum cholesterol and glucose levels in rats fed refined and less refined sugars and chromium. *J. Nutr. 97*, 237–242.
[100] Staub, H. W., Reussner, G., and Thiessen, R., Jr. (1969) Serum cholesterol reduction by chromium in hypercholesterolemic rats. *Science 166*, 746–747.
[101] Schroeder, H. A., and Balassa, J. J. (1965) Influence of chromium, cadmium, and lead on rat aortic lipids and circulating cholesterol. *Am. J. Physiol. 209*, 433–437.
[102] Roginski, E. E., and Mertz, W. (1969) Effects of chromium(III) supplementation on glucose and amino acid metabolism in rats fed a low protein diet. *J. Nutr. 97*, 525–530.
[103] Mertz, W., and Roginski, E. E. (1969) Effects of chromium(III) supplementation on growth and survival under stress in rats fed low protein diets. *J. Nutr. 97*, 531–536.
[104] O'Flaherty, E. J., and McCarty, C. P. (1978) Alterations of rat adipose tissue metabolism associated with dietary chromium supplementation. *J. Nutr. 108*, 321–328.
[105] Anderson, R. A., and Polansky, M. M. (1981) Dietary chromium deficiency. Effect on sperm count and fertility in rats. *Biol. Trace Elem. Res. 3*, 1–5.
[106] Striffler, J. S., Polansky, M. M., and Anderson, R. A. (1993) Dietary chromium enhances insulin secretion in perfused rat pancreas. *J. Trace Elem. Exp. Med. 6*, 75–81.
[107] Jain, R., Verch, R. L., Wallach, S., and Peabody, R. A. (1981) Tissue chromium exchange in the rat. *Am. J. Clin. Nutr. 34*, 2199–2204.
[108] Donaldson, D. L., Lee, D. M., Smith, C. C., and Rennert, O. M. (1985) Glucose tolerance and plasma lipid distributions in rats fed a high-sucrose, high-cholesterol, low-chromium diet. *Metabolism 34*, 1086–1093.
[109] Flatt, P. R., Juntti-Berggren, L., Berggren, P.-O., Gould, B. J., and Swanston-Flatt, S. K. (1989) Effects of dietary inorganic trivalent chromium (Cr) on the development of glucose homeostasis in rats. *Diabete Metabolisme 15*, 93–97.
[110] Striffler, J. S., Law, J. S., Polansky, M. M., Bhathena, S. J., and Anderson, R. A. (1995) Chromium improves insulin response to glucose in rats. *Metabolism 44*, 1314–1320.
[111] Lukaski, H. C. (1999) Chromium as a supplement. *Ann. Rev. Nutr. 19*, 279–302.
[112] Anderson, R. A. (1998) Recent advances in the clinical and biochemical manifestation of chromium deficiency in human and animal nutrition. *J. Trace Elem. Exp. Med. 11*, 241–250.
[113] Anderson, R. A. (1998) Effects of chromium on body composition and weight loss. *Nutr. Rev. 56*, 266–270.
[114] Anderson, R. A. (1998) Chromium, glucose intolerance and diabetes. *J. Am. Coll. Nutr. 17*, 548–555.
[115] Jeejeebhoy, K. N. (1999) The role of chromium in nutrition and therapeutics and as a potential toxin. *Nutr. Rev. 57*, 329–335.
[116] Mertz, W. (1993) Chromium in human nutrition: A review. *J. Nutr. 123*, 626–633.
[117] Veillon, C., and Patterson, K. Y. (1999) Analytical issues in nutritional chromium research. *J. Trace Elem. Exp. Med. 12*, 99–109.
[118] Anderson, R. A. (1987) Chromium in *Trace Elements in Human and Animal Nutrition* (Mertz, W., Ed.) Vol. 1, pp. 225–244, Academic Press, Orlando.
[119] Liu, V. J. K., and Morris, J. S. (1978) Relative chromium response as an indicator of chromium status. *Am. J. Clin. Nutr. 31*, 972–976.
[120] Offenbacher, E. G., and Pi-Sunyer, F. X. (1980) Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects. *Diabetes 29*, 919–925.

[121] Uusitupa, M., Mykkanen, L., Siitonen, O., Laakso, M., Sarlund, H., Kolehmainen, P., Rasanen, T., Kumpulainen, J., and Pyorala, K. (1992) Chromium supplementation in impaired glucose tolerance of elderly: Effects on blood glucose, plasma insulin, C-peptide and lipid levels. *Br. J. Nutr. 68*, 209–216.

[122] Roeback, J. R., Jr, Khin Mae, H., Chambless, L. E., and Fletcher, R. H. (1991) Effects of chromium supplementation on serum high-density lipoprotein cholesterol levels in men taking beta-blockers. *Ann. Intern. Med. 115*, 917–924.

[123] Bourn, D. M., Gibson, R. S., Martinez, O. G., and MacDonald, A. C. (1986) The effect of chromium supplementation on serum lipid levels in a selected sample of Canadian postmenopausal women. *Biol. Trace Elem. Res. 9*, 197–205.

[124] Hopkins, Jr, L. L., Ransome-Kuti, O., and Majaj, A. S. (1968) Improvement of impaired carbohydrate metabolism by chromium(III) in malnourished infants. *Am. J. Clin. Nutr. 21*, 203–211.

[125] Levine, R. A., Streeten, D. H. P., and Doisy, R. J. (1968) Effects of oral chromium supplementation on the glucose tolerance of elderly human subjects. *Metabolism 17*, 114–125.

[126] Glinsmann, W. H., and Mertz, W. (1966) Effect of trivalent chromium on glucose tolerance. *Metabolism 15*, 510–520.

[127] Carter, J. P., Kattab, A., Abd-el-Hadi, K., Davis, J. T., el Gholmy, A., and Patwardhan, V. N. (1968) Chromium(III) in hypoglycemia and in impaired glucose utilization in kwashiorkor. *Am. J. Clin. Nutr. 21*, 195–202.

[128] Gurson, C. T., and Saner, G. (1971) Effect of chromium on glucose utilization in marasmic protein-calorie malnutrition. *Am. J. Clin. Nutr. 24*, 1313–1319.

[129] Riales, R., and Albrink, M. J. (1981) Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high-density lipoprotein of adult men. *Am. J. Clin. Nutr. 34*, 2670–2678.

[130] Offenbacher, K. G., Rinko, C. J., and Pi-Sunyer, X. (1985) The effects of inorganic chromium and brewer's yeast on glucose tolerance, plasma lipids, and plasma chromium in elderly subjects. *J. Clin. Nutr. 42*, 454–461.

[131] Anderson, R. A., Polansky, M. M., Bryden, N. A., Roginski, E. E., Mertz, W., and Glinsmann, W. (1983) Chromium supplementation of human subjects: Effects on glucose, insulin, and lipid variables. *Metabolism 32*, 894–899.

[132] Potter, J. F., Levin, P., Anderson, R. A., Freiberg, J. M., Andres, R., and Elahi, D. (1985) Glucose metabolism in glucose-intolerant older people during chromium supplementation. *Metabolism 34*, 199–204.

[133] Martinez, O. B., MacDonald, A. C., Gibson, R. S., and Bourn, D. (1985) Dietary chromium and effect of chromium supplementation on glucose tolerance of elderly Canadian women. *Nutr. Res. 5*, 609–620.

[134] Urberg, M., and Zemel, M. B. (1987) Evidence for synergism between chromium and nicotinic acid in the control of glucose tolerance in elderly humans. *Metabolism 36*, 896–899.

[135] Urberg, M., Benyi, J., and John, R. (1988) Hypocholesterolemic effects of nicotinic acid and chromium supplementation. *J. Fam. Pract. 27*, 603–606.

[136] Wang, M. M., Fox, E. A., Stoecker, B. J., Menendez, C. E., and Chan, S. B. (1989) Serum cholesterol of adults supplemented with brewer's yeast or chromium chloride. *Nutr. Res. 9*, 989–998.

[137] Lefavi, R. G., Wilson, G. D., Keith, R. E., Anderson, R. A., Blessing, D. L., Hames, C. G., and McMillan, J. L. (1993) Lipid-lowering effect of a dietary chromium (III)-nicotinic acid complex in male athletes. *Nutr. Res. 13*, 239–249.

[138] Wilson, B. E., and Gondy, A. (1995) Effects of chromium supplementation on fasting insulin levels and lipid parameters in healthy, non-obese young subjects. *Diabetes Res. Clin. Prac. 28*, 179–184.

[139] Thomas, V. L. K., and Gropper, S. S. (1996) Effect of chromium nicotinic acid supplementation on selected cardiovascular disease risk factors. *Biol. Trace Elem. Res. 55*, 297–305.
[140] Anderson, R. A., Polansky, M. M., Bryden, N. A., and Canary, J. J. (1991) Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am. J. Clin. Nutr. 54*, 909–916.
[141] Abraham, A. S., Brooks, B. A., and Eylath, U. (1992) The effects of chromium supplementation on serum glucose and lipids in patients with and without non-insulin-dependent diabetes. *Metabolism 41*, 768–771.
[142] Sherman, L., Glennon, J. A., Brech, W. J., Klomberg, G. H., and Gordon, E. S. (1968) Failure of trivalent chromium to improve hyperglycemia in diabetes mellitus. *Metabolism 17*, 439–442.
[143] Anderson, R. A., Polansky, M. M., Bryden, N. A., Bhathena, S. J., and Canary, J. J. (1987) Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. *Metabolism 36*, 351–355.
[144] Anderson, R. A. (1995) Chromium and parenteral nutrition. *Nutrition 11*, 83–86.
[145] Brown, R. O., Forloines-Lynn, S., Cross, R. E., and Heizer, W. D. (1986) Chromium deficiency after long-term total parenteral nutrition. *Dig. Dis. Sci. 31*, 661–664.
[146] Verhage, A. H., Cheong, W. K., and Jeejeebhoy, K. N. (1996) Neurologic symptoms due to possible chromium deficiency in long-term parenteral nutrition that closely mimic metronidazole-induced syndromes. *JPEN 20*, 123–127.
[147] Jeejeebhoy, K. N., Chu, R. C., Marliss, E. B., Greenberg, G. R., and Bruce-Robertson, A. (1977) Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long term total parenteral nutrition. *Am. J. Clin. Nutr. 30*, 531–538.
[148] Freund, H., Atamian, S., and Fischer, J. E. (1979) Chromium deficiency during total parenteral nutrition. *JAMA 241*, 496–498.
[149] Scully, R. E., Mark, E. J., McNeely, W. F., and McNeely, B. U. (1990) Case records of the Massachusetts General Hospital: Case 12-1990. *New Engl. J. Med. 322*, 829–841.
[150] Jeejeebhoy, K. N. (1999) Chromium and parenteral nutrition. *J. Trace Elem. Exp. Med. 12*, 85–89.
[151] Stearns, D. M. (2000) Is chromium a trace essential element? *Biofactors 11*, 149–162.
[152] Anderson, R. A., and Kozlovsky, A. S. (1985) Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr. 41*, 1177–1183.
[153] Anderson, R. A., and Polansky, M. M. (1995) Dietary and metabolite effects on trivalent chromium retention and distribution in rats. *Biol. Trace Elem. Res. 50*, 97–108.
[154] Morris, B. W., MacNeil, S., Stanley, K., Gray, T. A., and Fraser, R. (1993) The inter-relationship between insulin and chromium in hyperinsulinaemic euglycaemic clamps in healthy volunteers. *J. Endocrin. 139*, 339–345.
[155] Morris, B. W., Gray, T. A., and Macneil, S. (1993) Glucose-dependent uptake of chromium in human and rat insulin-sensitive tissues. *Clin. Sci. 84*, 477–482.
[156] Anderson, R. A., Polansky, M. M., Bryden, N. A., Roginski, E. E., Patterson, K. Y., Veillon, C., and Glinsmann, W. (1982) Urinary chromium excretion of human subjects: Effects of chromium supplementation and glucose loading. *Am. J. Clin. Nutr. 36*, 1184–1193.
[157] Anderson, R. A., Polansky, M. M., Bryden, N., Roginski, E. E., Patterson, K. Y., and Reamer, D. C. (1982) Effect of exercise (running) on serum glucose, insulin, glucagon, and chromium excretion. *Diabetes 31*, 212–216.
[158] Kozlovsky, A. S., Moser, P. B., Reiser, S., and Anderson, R. A. (1986) Effects of diets high in simple sugars on urinary chromium losses. *Metabolism 35*, 515–518.
[159] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Reiser, S. (1990) Urinary chromium excretion and insulinogenic properties of carbohydrates. *Am. J. Clin. Nutr. 51*, 864–868.

[160] Morris, B. W., Blumsohn, A., Mac Neil, S., and Gray, T. A. (1992) The trace element chromium – A role in glucose homeostasis. *Am. J. Clin. Nutr. 55*, 989–991.
[161] Morris, B. W., Griffiths, H., and Kemp, G. J. (1988) Effect of glucose loading on concentrations of chromium in plasma and urine of healthy adults. *Clin. Chem. 34*, 1114–1116.
[162] National Research Council (1980) *Recommended Dietary Allowances, 9th Ed. Report of the Committee on Dietary Allowances, Division of Biological Sciences, Assembly of Life Science, Food and Nutrition Board, Commission on Life Science, National Research Council*. National Academy Press, Washington, D. C.
[163] National Research Council (1989) *Recommended Dietary Allowances, 10th Ed. Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Commission on Life Science, National Research Council*. National Academy Press, Washington, D C
[164] Hunt, C. D., and Stoecker, B. J. (1996) Deliberations and evaluations of the approaches, endpoints and paradigms for boron, chromium and fluoride dietary recommendations. *J. Nutr. 126*, 2441S–2451S.
[165] Anderson, R. A. (1994) Nutritional and toxicological aspects ofchromium intake: An overview in *Risk Assessment of Essential Elements* (Mertz, W., Abernathy, C. O., and Olin, S. S., Eds) pp 187–196, ISLI Press, Washington, D. C.
[166] Anderson, R. A., Bryden, N. A., and Polansky, M. M. (1993) Dietary intake of calcium, chromium, copper, iron, magnesium, manganese, and zinc: Duplicate plate values corrected using derived nutrient intake. *J. Am. Diet. Assoc. 93*, 462–464.
[167] Gibson, R. S., and Scythes, C. A. (1984) Chromium, selenium, and other trace element intakes of a selected sample of Canadian premenopausal women. *Biol. Trace Elem. Res. 6*, 105–116.
[168] Offenbacher, E. G., Spencer, H., Dowling, H. J., and Pi-Sunyer, F. X. (1986) Metabolic chromium balances in men. *Am. J. Clin. Nutr. 44*, 77–82.
[169] Bunker, V. W., Lawson, M. S., Delves, H. T., and Clayton, B. E. (1984) The uptake and excretion of chromium by the elderly. *Am. J. Clin. Nutr. 39*, 797–802.
[170] National Research Council (2002) *Dietary Reference Intakes for vitamin A, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A report of the Panel on Micronutrients, Subcommittee on Upper Reference Levels of Nutrients and of Interpretations and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes*. National Academy of Sciences, Washington, D. C.
[171] Hathcock, J. N. (1996) Safety limits for nutrients. *J. Nutr. 126*, 2386S–2389S.
[172] Yamamoto, A., Wada, O., and Suzuki, H. (1987) Separation of biologically active chromium complex from cow colostrum. *Tohoku J. Exp. Med. 152*, 211–219.
[173] National Research Council (1997) The role of chromium in animal nutrition, National Academy Press, Washington, D. C.
[174] Yamamoto, A., Wada, O., and Suzuki, H. (1988) Purification and properties of biologically active chromium complex from bovine colostrum. *J. Nutr. 118*, 39–45.
[175] Yamamoto, A., Wada, O., and Manabe, S. (1989) Evidence that chromium is an essential factor for biological activity of low-molecular weight Cr-binding substance. *Biochem. Biophys. Res. Commun. 163*, 189–193.
[176] Yamamoto, A., Wada, O., and Ono, T. (1987) Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. *Eur. J. Biochem. 165*, 627–631.
[177] Yamamoto, A., Wada, O., and Ono, T. (1984) Distribution and chromium-binding capacity of a low-molecular-weight, chromium-binding substance in mice. *J. Inorg. Biochem. 22*, 91–102.
[178] Wada, O., Wu, G. Y., Yamamoto, A., Manabe, S., and Ono, T. (1983) Purification and chromium-excretory function of low-molecular-weight, chromium-binding substances from dog liver. *Environ. Res. 32*, 228–239.

[179] Sun, Y., Mallya, K., Ramirez, J., and Vincent, J. B. (1999) The biomimetic $[Cr_3O(O_2CCH_2CH_3)_6(H2O)_3]^+$ decreases plasma cholesterol and triglycerides in rats: Towards chromium-containing therapeutics. *J. Biol. Inorg. Chem. 4*, 838–845.

[180] Wada, O., Manabe, S., Yamaguchi, N., Ishikawa, S., and Yanagisawa, H. (1983) Low-molecular-weight, chromium-binding substance in rat lungs and its possible role in chromium movement. *Ind. Health 21*, 35–41.

[181] Cannon, R. D., and White, R. P. (1988) Chemical and physical properties of triangular bridged metal complexes. *Prog. Inorg. Chem 36*, 195–297.

[182] Nagi, M., Harton, A., Donald, S., Lee, Y. S., Sabat, M., O'Connor, C. J., and Vincent, J. B. (1995) An unsymmetric trinuclear chromium(III) oxo carboxylate assembly: structure and characterization of $Cr_3O(O_2CPh)_4(8\text{-hqn})_3 1.25CH_2Cl_2$. *Inorg. Chem. 34*, 3813–3820.

[183] Harton, A., Terrell, K., Huffman, J. C., MacDonald, C., Beatty, A., Li, S., O'Connor, C., and Vincent, J. B. (1997) Synthesis and characterization of novel oxo-bridged dinuclear and hydroxo-bridged trinuclear chromium(III) Assemblies. *Inorg. Chem. 36*, 4875–4882.

[184] Bino, A., Chayat, R., Pedersen, E., and Schneider, A. (1991) A new tetranuclear chromium(III) complex with a $[Cr_4O_2]$ core: synthesis, structure, and magnetic properties of $[Cr_4O_2(O_2CCH_3)_7(bpy)_2]PF_6$ (bpy = 2,2'-bipyridine). *Inorg. Chem. 30*, 856–858.

[185] Donald, S., Terrell, K., Vincent, J. B., and Robinson, K. D. (1995) Modelling chromium biochemistry: Synthesis and characterization of $[NEt_3H][Cr(III)_4O_2(O_2CPh)_7(pic)_2]\cdot CH_2Cl_2$ (Hpic = Picolinic acid). *Polyhedron 14*, 971–976.

[186] Ellis, T., Glass, M., Harton, A., Folting, K., Huffman, J. C., and Vincent, J. B. (1994) Synthetic models for low-molecular-weight chromium-binding substance: synthesis and characterization of oxo-bridged tetranuclear chromium(III) assemblies. *Inorg. Chem. 33*, 5522–5527.

[187] Wacker, W. E. C., and Vallee, B. L. (1959) Chromium, manganese, nickel, and other metals in RNA. *Fed. Proc. 18*, 345.

[188] Wacker, W. E. C., and Vallee, B. L. Nature 184 (1959) Chromium ribonucleoprotein from bovine liver. *Nature 184*, 1399.

[189] Okada, S., Ohba, H., and Taniyama, M. (1981) Alterations in ribonucleic acid synthesis by Chromium(III). *J. Inorg. Biochem. 15*, 223–231.

[190] Okada, S., Tsukada, H., and Tezuka, M. (1989) Effect of Chromium(III) on nuclear RNA synthesis. *Biol. Trace Elem. Res. 21*, 35–39.

[191] Okada, S., Tsukada, H., and Ohba, H. (1984) Enhancement of nucleolar RNA synthesis by chromium(III) in regenerating rat liver. *J. Inorg. Biochem. 21*, 113–124.

[192] Ohba, H., Suketa, Y., and Okada, S. (1986) Enhancement of in vitro ribonucleic acid synthesis on chromium(III)-bound chromatin. *J. Inorg. Biochem. 27*, 179–189.

[193] Okada, S., Taniyama, M., and Ohba, H. (1982) Mode of enhancement in ribonucleic acid synthesis directed by chromium (III)-bound deoxyribonucleic acid. *J. Inorg. Biochem. 17*, 41–49.

[194] Morris, B. W., Kemp, G. J., and Hardisty, C. A. (1985) Plasma chromium and chromium excretion in diabetes. *Clin. Chem. 31*, 334–335.

[195] Morris, B. W., Griffiths, H., and Kemp, G. J. (1988) Correlations between abnormalities in chromium and glucose metabolism in a group of diabetics. *Clin. Chem. 34*, 1525–1526.

[196] Morris, B. W., Macneil, S., Hardisty, C. A., Heller, S., Burgin, C., and Gray, T. A. (1999) Chromium homeostasis in patients with type II (NIDDM) diabetes. *J. Trace Elem. Med. Biol. 13*, 57–61.

[197] Anderson, R. A., Bryden, N. A., and Polansky, M. M. (1985) Serum chromium of human subjects: Effects of chromium supplementation and glucose. *Am. J. Clin. Nutr. 41*, 571–577.

[198] Rabinowitz, M. B., Gonick, H. C., Levine, S. R., and Davidson, M. B. (1983) Clinical trial of chromium and yeast supplements on carbohydrate and lipid metabolism in diabetic men. *Biol. Trace Elem. Res. 5*, 449–466.

[199] Uusitupa, M. I. J., Kumpulainen, J. T., Voutilainen, E., and etal. (1983) Effect of inorganic chromium supplementation on glucose tolerance, insulin response, and serum lipids in noninsulin-dependent diabetics. *Am. J. Clin. Nutr. 38*, 404–410.

[200] Lee, N. A., and Reasner, C. A. (1994) Beneficial effect of chromium supplementation on serum triglyceride levels in NIDDM. *Diabetes Care 17*, 1449–1452.

[201] Nath, R., Minocha, J., Lyall, V., Sunder, S., Kumar, V., Kapoor, S., and Dhar, K. L. (1979) Synthesis and in vitro insulin potentiating activity of glutathione-nicotinic-acid-chromium complex in *Chromium in Nutrition and Metabolism* (Shapcott, D., and Hubert, J., Eds.) pp. 213–222, Elsevier, North Holland.

[202] Elias, A. N., Grossman, M. K., and Valenta, L. J. (1984) Use of the artificial beta cell (ABC) in the assessment of peripheral insulin sensitivity: Effect of chromium supplementation in diabetic patients. *Gen. Pharm. 15*, 535–539.

[203] Mossop, R. T. (1983) Effects of chromium(III) on fasting blood glucose, cholesterol and cholesterol HDL levels in diabetics. *Cent. Afr. J. Med. 29*, 80–82.

[204] Ravina, A., Slezak, L., Rubal, A., and Mirsky, N. (1995) Clinical use of the trace element chromium(III) in the treatment of diabetes mellitus. *J. Trace Elem. Exp. Med. 8*, 183–190.

[205] Jovanovic-Peterson, L., Guiterry, M., and Peterson, C. M. (1996) Chromium supplementation for gestational diabetic women (GDM) improves glucose tolerance and decreases hyperinsulinemia. *Diabetes 43*, 337A.

[206] Anderson, R. A., Cheng, N., Bryden, N. A., Polansky, M. M., Cheng, N., Chi, J., and Feng, J. (1997) Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.

[207] Borel, J. S., Majerus, T. C., Polansky, M. M., and et al. (1984) Chromium intake and urinary chromium excretion of trauma patients. *Biol. Trace Elem. Res. 6*, 317–326.

[208] Anderson, R. A., Borel, J. S., Polansky, M. M., Bryden, N. A., Majerus, T. C., and Moser, P. B. (1988) Chromium intake and excretion of patients receiving total parenteral nutrition: Effects of supplemental chromium. *J. Trace Elem. Exp. Med. 1*, 9–18.

[209] Anderson, R. A., Polansky, M. M., and Bryden, N. A. (1984) Strenuous running. Acute effects on chromium, copper, zinc, and selected clinical variables in urine and serum of male runners. *Biol. Trace Elem. Res. 6*, 327–336.

[210] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Thorp, J. W. (1991) Effects of carbohydrate loading and underwater exercise on circulating cortisol, insulin and urinary losses of chromium and zinc. *Eur. J. Appl. Physiol. Occup. Physiol. 63*, 146–150.

[211] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Deuster, P. A. (1988) Exercise effects on chromium excretion of trained and untrained men consuming a constant diet. *J. Appl. Physiol. 64*, 249–252.

[212] Clarkson, P. M. (1997) Effects of exercise on chromium levels. Is supplementation required? *Sports Med. 23*, 341–349.

[213] Morris, B., MacNeil, S., Fraser, R., and Gray, T. (1995) Increased urine chromium excretion in normal pregnancy. *Clin. Chem. 41*, 1544–1545.

[214] Jovanovic, L., Gutierrez, M., and Peterson, C. M. (1999) Chromium supplementation for women with gestational diabetes mellitus. *J. Trace Elem. Exp. Med. 12*, 91–97.

[215] Anderson, R. A., Polansky, M. M., Bryden, N. A., and etal. (1983) Effects of chromium supplementation on urinary Cr excretion of human subjects and correlation of Cr excretion with selected clinical parameters. *J. Nutr. 113*, 276–281.

[216] Dowling, H. J., Offenbacher, E. G., and Pi-Sunyer, F. X. (1989) Absorption of inorganic, trivalent chromium from the vascularly perfused rat small intestine. *J. Nutr. 119*, 1138–1145.

[217] Donaldson, R. M., Jr, and Barreras, R. F. (1966) Intestinal absorption of trace quantities of chromium. *J. Lab. Clin. Med. 68*, 484–493.

[218] Mertz, W., and Roginski, E. E. (1971) Chromium metabolism & the glucose tolerance factor in *Newer Trace Elements in Nutrition* (Mertz, W., and Cornatzer, W. E., Eds.) pp. 123–153, Dekker Press, New York.

[219] Chen, N. S. C., Tsai, A., and Dyer, I. A. (1973) Effect of chelating agents on chromium absorption in rats. *J. Nutr. 103*, 1182–1186.

[220] Dowling, H. J., Offenbacher, E. G., and Pi-Sunyer, F. X. (1990) Effects of amino acids on the absorption of trivalent chromium and its retention by regions of the rat small intestine. *Nutr. Res. 10*, 1261–1271.

[221] Seaborn, C. D., and Stoccker, B. J. (1992) Effects of ascorbic acid depletion and chromium status on retention and urinary excretion of chromium. *Nutr. Res. 12*, 1229–1234.

[222] Keim, K. S., Stoecker, B. J., and Henley, S. (1987) Chromium status of the rat as affected by phytate. *Nutr. Res. 7*, 253–263.

[223] Hopkins, J., L. L., and Schwarz, K. (1964) Chromium (III) binding to serum proteins, specifically siderophilin. *Biochim. Biophys. Acta 90*, 484–491.

[224] Borguet, F., Cornelis, R., and Lameire, N. (1990) Speciation of chromium in plasma and liver tissue of endstage renal failure patients on continuous ambulatory peritoneal dialysis. *Biol. Trace Elem. Res. 26–27*, 449–460.

[225] Borguet, F., Cornelis, R., Delanghe, J., Lambert, M.-C., and Lameire, N. (1995) Study of the chromium binding in plasma of patients on continuous ambulatory peritoneal dialysis. *Clin. Chimica. Acta 238*, 71–84.

[226] Dowling, H. J., Offenbacher, E. G., and Pi-Sunyer, F. X. (1990) Effects of plasma transferrin and albumin on the absorption of trivalent chromium. *Nutr. Res. 10*, 1251–1260.

[227] Brock, J. H. (1985) Transferrins in *Metalloproteins* (Harrison, P. M., Ed.) Vol. 2, pp. 183–262, MacMillan, London.

[228] Aisen, P., Aasa, R., and Redfield, A. G. (1969) The chromium, manganese, and cobalt complexes of transferrin. *J. Biol. Chem. 244*, 4628–4633.

[229] Tan, A. T., and Woodworth, R. C. (1969) Ultraviolet difference spectral studies of conalbumin complexes with transition metal ions. *Biochemistry 8*, 3711–3716.

[230] Harris, D. C. (1977) Different metal-binding properties of the two sites of human transferrin. *Biochemistry 16*, 560–564.

[231] Ainscough, E. W., Brodie, A. M., Plowman, J. E., and etal. (1980) Studies on human lactoferrin by electron paramagnetic resonance, fluorescence, and resonance Raman spectroscopy. *Biochemistry 19*, 4072–4079.

[232] Ainscough, E. W., Brodie, A. M., and Plowman, J. E. (1979) The chromium, manganese, cobalt and copper complexes of human lactoferrin. *Inorg. Chim. Acta 33*, 149–153.

[233] Moshtaghie, A. A., Ani, M., and Bazrafshan, M. R. (1992) Comparative binding study of aluminum and chromium to human transferrin: Effect of iron. *Biol. Trace Elem. Res. 32*, 39–46.

[234] Ani, M., and Moshtaghie, A. A. (1992) The effect of chromium on parameters related to iron metabolism. *Biol. Trace Elem. Res. 32*, 57–64.

[235] Sargent, T., III, Lim, T. H., and Jenson, R. L. (1979) Reduced chromium retention in rations with hemochromatosis, a possible basis of hemochromatotic diabetes. *Metabolism 28*, 70–79.

[236] Hopkins, L. L., Jr (1965) Distribution in the rat of physiological amounts of injected $Cr^{51}$(III) with time. *Am. J. Physiol. 209*, 731–735.

[237] Davis-Whitenack, M. L., Adeleye, B. O., Rolf, L. L., and Stoecker, B. J. (1996) Biliary excretion of $^{51}$chromium in bile-duct cannulated rats. *Nutr. Res. 16*, 1009–1015.

[238] Manzo, L., Di Nucci, A., Edel, J., and etal. (1983) Biliary and gastrointestinal excretion of chromium after administration of Cr-III and Cr-VI in rats. *Res. Commun. Chem. Pathol. Pharmacol. 42*, 113–125.

[239] Donaldson, D. L., Smith, C. C., and Yunice, A. A. (1984) Renal excretion of chromium-51 chloride in the dog. *Am. J. Physiol. 15*, F870–F878.
[240] Collins, R. J., Fromm, P. O., and Collings, W. D. (1961) Chromium excretion in the dog. *Am. J. Physiol. 201*, 795–798.
[241] Wallach, S., and Verch, R. L. (1983) Radiochromium conservation and distribution in diuretic states. *J. Am. Coll. Nutr. 2*, 163–172.
[242] Onkelinx, C. (1977) Compartment analysis of metabolism of chromium(III) in rats of various ages. *Am. J. Physiol. 232*, E478–E484.
[243] Lim, T. H., Sargent T., III, and Kusubov, N. (1983) Kinetics of trace element chromium(III) in the human body. *Am. J. Physiol. 13*, R445–R454.
[244] Kraszeski, J. L., Wallach, S., and Verch, R. L. (1979) Effect of insulin on radiochromium distribution in diabetic rats. *Endocrinology 104*, 881–885.
[245] Wallach, S., and Verch, R. L. (1986) Radiochromium distribution in aged rats. *J. Am. Coll. Nutr. 5*, 291–298.
[246] Fernandez-Lopez, J.-A., Esteve, M., Rafecas, I., Remesar, X., and Alemany, M. (1994) Management of dietary essential metals (iron, copper, zinc, chromium, and manganese) by Wistar and Zucker obese rats fed a self-selected high-energy diet. *BioMetals 7*, 117–129.

# Part I

# Chromium as a nutrient and nutritional supplement

This page intentionally left blank

*Chapter 2*

# Basis for dietary recommendations for chromium

**Barbara J. Stoecker**

Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK 74078

## INTRODUCTION

The development of the Dietary Reference Intakes (DRIs) was a collaborative effort between American and Canadian scientists; parent organizations were the Food and Nutrition Board of the Institute of Medicine and Health Canada. The DRI for chromium was established in 2001 under the auspices of the Food and Nutrition Board's Standing DRI Committee and involved extensive input from the Panel on Micronutrients, the Subcommittee on Upper Reference Levels of Nutrients, and the Subcommittee on the Interpretation and Uses of the DRIs [1].

Because of the paucity of available data on metabolic responses to usual dietary intakes of chromium, recommendations were established as *Adequate Intakes (AI)* for different age/gender groups rather than as *Estimated Average Requirements (EAR)* with resulting *Recommended Dietary Allowances (RDA)*. Based on policy and guidelines for establishing the DRIs, if data are insufficient for establishing a nutrient intake estimated to meet the needs of half of the healthy individuals in a life stage and gender group (EAR), it is not possible to establish an RDA. There was only one study in which participants were fed a controlled low-chromium diet [2], and this study did not allow the determination of the point at which half of the participants were chromium deficient. Therefore, the chromium recommendation is expressed as an AI and is based on the available data on chromium in well-balanced diets.

The chromium AIs represent a substantial decrease from the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for chromium first established in 1980 [3] and retained in the 10th edition of the Recommended Dietary Allowances published in 1989 [4]. In the late 1970s when the first ESADDI for chromium was established, almost all data on chromium in blood, urine, and foods were erroneous. Chromium concentrations in biological samples are too low to be determined by flame atomic spectrophotometer and graphite furnace atomic absorption spectrophotometers were not widely available; furthermore, prior to 1980, samples were typically contaminated during collection. There was a general lack of awareness of the precautions needed for handling samples for ultratrace element analyses. Blood samples acquired chromium by being

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

drawn through stainless steel needles and food samples were contaminated by contact with stainless steel blender blades used in homogenizing samples. Samples being ashed were typically contaminated by chromium from the acids used in the ashing process or from the laboratory environment [5–7]. In late 1978, Guthrie and colleagues [8] published a very important paper on background correction problems affecting the determination of chromium in urine by graphite furnace atomic absorption spectrophotometry. Conventional deuterium background correction was inadequate for assessment of urinary chromium; consequently, even laboratories doing very careful trace element work were severely overestimating urinary chromium.

At the time it was established, the ESADDI of 50–200 μg/day for chromium seemed reasonable based on available studies from the 1970s and before that reported urinary chromium excretion to be 1–3 μg/day. An early study using $^{51}Cr$ had found chromium absorption to be 1–2% of the dose [9]. If chromium absorption from food were similar to the absorption of $^{51}CrCl_3$, then the ESADDI accounted for the chromium in urine.

Since that time, graphite furnace atomic absorption spectrophotometry with Zeeman background correction and stable isotope methodology have been used to acquire accurate data on chromium in blood, urine, and milk. There is consensus that urinary Cr excretion in the United States is in the range of 0.2–0.3 μg/day, that is approximately 5–10-fold lower than good laboratories were reporting in the 1970s. The chromium concentration in human milk is extremely low as is the chromium concentration in most foods. Unfortunately, these low levels make contamination a continuing analytical problem and there remains a paucity of data on chromium in the clinical setting. Furthermore, analysis of chromium in the food supply has been done in only a few locations and countries, so there is not a good basis for evaluating possible differences in chromium in different regions in the United States or internationally.

The AIs established in 2001 are shown in the following table. The studies providing the basis for the current chromium AIs will be discussed below.

## DIETARY REFERENCE INTAKE VALUES FOR CHROMIUM BY AGE AND GENDER

The following table details the AI values for chromium [1]:

| Life stage group | AI (μg/day) | |
|---|---|---|
| | Male | Female |
| 0–6 months | 0.2 | 0.2 |
| 7–12 months | 5.5 | 5.5 |
| 1–3 years | 11 | 11 |
| 4–8 years | 15 | 15 |
| 9–13 years | 25 | 21 |
| 14–18 years | 35 | 24 |
| 19–50 years | 35 | 25 |
| ≥51 years | 30 | 20 |

| | |
|---|---|
| *Pregnancy* | |
| ≤18 years | 29 |
| 19–50 years | 30 |
| *Lactation* | |
| ≤18 years | 44 |
| 19–50 years | 45 |

The AI for 0–6 months was based on the average content of chromium in human milk while the value for 7–12 months includes estimates from human milk and complementary foods. For adults, the average chromium content in well-balanced diets was taken to be 13.4 μg/1000 kcal and the average energy intake was based on data from NHANES III [10]. The AIs for children were extrapolated from the adult AI; levels for pregnancy and lactation were extrapolated from the appropriate age group based on body weight with additional increments for average human milk content of chromium.

## BASIS FOR CONSIDERATION OF CHROMIUM AS AN ESSENTIAL NUTRIENT

Chromium has been identified as an element that potentiates insulin action both in vivo and in vitro [11]. In 1957, Schwarz and Mertz reported that a substance in porcine kidney restored impaired glucose tolerance in rats [12]. Subsequently, Jeejeebhoy and colleagues [13] reported dramatic improvements in glucose regulation with chromium supplementation in a patient receiving total parenteral nutrition (TPN). This woman had received TPN for more than 3 years when she unexpectedly lost weight and displayed clinical signs of diabetic neuropathy. Furthermore, her plasma glucose levels, 2 hours after the end of her TPN infusion, were significantly elevated to 132 mg/dL. Insulin was given in increasing amounts until plasma glucose was normalized which required 45 units of crystalline zinc insulin. After various other potential sources of glucose intolerance were considered, 250 μg of chromium was infused daily for 2 weeks. This infusion produced substantial clinical improvement and a sharp fall in plasma glucose concentrations. The patient gained weight and no longer required exogenous insulin to maintain normal plasma glucose levels. The patient continued on an infusion of 20 μg Cr/day and a year and a half later still maintained normal glucose levels and reported no neuropathy. This and other early chromium studies have been comprehensively reviewed by several investigators [14–17].

## DATA LIMITATIONS FOR ESTABLISHING AN EAR

The data from the patient receiving TPN are very interesting, but at present there is no practical clinical test to specifically identify chromium deficiency in orally fed people. It is not surprising that many chromium supplementation studies have been equivocal, because it is not possible to specifically select only those subjects who are chromium deficient to include in a study. One would not expect beneficial effects

of supplementation for persons not deficient in a nutrient. Lack of a specific indicator of chromium status, in addition to the contamination problems associated with chromium measurement [6, 7, 14], has discouraged many investigators from studying chromium and has decreased enthusiasm among funding agencies for supporting their work.

Establishing an EAR requires identification of a level of dietary intake that meets the needs of half of the individuals being evaluated (as assessed by the selected indicator). There were no studies with chromium that systematically depleted individuals of chromium until a functional parameter was abnormal. Only one study fed subjects a low-chromium diet; in that study urinary chromium decreased but with the relatively small sample size this numerical change was not significant [2]. Without the ability to define an EAR, the only choice available to the panel was to base their recommendation on available dietary data.

## REPORTED CHROMIUM CONCENTRATIONS IN FOODS

Dietary chromium intakes reported before 1977 are at least three times higher than those reported more recently. Valid data on the chromium content of foods are very limited. This is primarily because of the difficulty in collecting and analyzing chromium accurately in food samples [6, 7, 18]. In earlier analyses, much of the reported chromium was acquired through contamination in sampling, sample homogenization, and analysis.

Little is known about variation in chromium concentration in plants grown in different areas of North America. Cary and Kubota analyzed samples collected from sites in Maryland, North Carolina, and northern California. Plant samples taken from areas with high-chromium soils contained higher concentrations of chromium than plants grown on low-chromium soils. Nonetheless, the authors noted that some of this chromium was apparently due to contamination of the plant material by soil chromium [19], and samples were ground in a Wiley mill fitted with a stainless steel screen which would also create the potential of sample contamination with chromium.

Most staple foods apparently provide less than 10 g/kg chromium [7]. However, a number of developing countries have reported higher dietary intakes than those reported by trace element laboratories in Scandinavian countries and in the US raising questions of regional differences or contamination problems.

With the exception of sugar which is often very low in chromium, processed foods generally are higher in chromium content than fresh foods [7]. Offenbacher and Pi-Sunyer reported on release of chromium from stainless steel into water and fruit juices [20]. The ability of water to stimulate chromium release from stainless steel was primarily dependent on pH. All processed fruit juices contained higher amounts of chromium than hand-squeezed juices, but the amount of chromium taken up in the acid juices did not parallel their pH. More chromium was taken up by juices than by the acidified water perhaps due to the characteristic organic acids in the juices. Amounts of chromium released into juices were 3–5 μg/100 mL which could make important contributions to overall chromium intake [20].

Anderson and coworkers analyzed chromium in breakfast cereals and found concentrations ranging from 3 to 701 ng/g. These cereals would then provide 0.25–30.7 μg Cr/serving. Many provided more than 5 μg Cr/serving and ready-to-eat bran cereals tended to be higher than others [21]. Variation seemed to be due to contamination from processing and/or fortification. Contaminating chromium may help to meet human chromium requirement.

A number of individual food items with chromium concentrations were reported by the Beltsville Human Nutrition Research Center group [22]. Dairy products were poor sources of chromium. Milk at <0.12 μg Cr/serving added little to the daily chromium intake. Meats, poultry, and fish, except for one processed luncheon meat analyzed, were generally low in chromium with one serving providing 0.5–3.6 μg chromium. The turkey ham, on the other hand, had 10.4 μg chromium in a 3-ounce serving. Because unprocessed turkey had <2 μg Cr/serving, it would seem that much of the chromium in turkey ham came from the processing [22].

Chromium content of fruits and vegetables varied from 0.1 to 22 μg/serving. A serving of broccoli at 22 μg/serving provided more than three-fold more Cr than any other vegetable or fruit tested. The authors indicated follow-up tests were being done, but no additional papers have been published in this area [22]. Perhaps the broccoli retained traces of soil; earlier studies had noted the difficulty in avoiding contamination of some plant samples with soil [19].

The brand of barbeque sauce tested contained 1.73 μg Cr/tablespoon. Some spices have high chromium concentrations, but because the usual diet contains only a very small amount of these substances, they do not make a major contribution to the overall daily chromium intake [22].

Chromium concentrations in beer were found to vary widely, from 0.48 to 56 ng/mL. Chromium in 8 brands of beer was below 2 ng/mL, 11 beers ranged from 2 to 10 ng/mL, and 8 brands of beer had chromium concentrations above 10 ng/mL. Chromium concentration in the brewing water was 0.5 ng/mL or less. Based on the relative amounts of the components present in beer, the authors estimated the following contributions per liter to the overall chromium content: water, 0.5 μg; corn grits, 0.3 μg; malt, 4.8 μg; and hops 1.2 μg for a total of ~6.8 μg/L of beer. Additional chromium came from unknown sources, but some release from stainless steel may have been a possibility [22].

Wine is also a potential source of small amounts of chromium. Although mean concentrations were similar for red and white wines tested in France, the red wine ranged from 7 to 90 μg Cr/L and the white wine ranged from 7 to 44 μg Cr/L. The chromium concentrations of 94% of all wines tested were <40 μg/L. The authors estimated the contribution of wine consumption to total Cr dietary intake for the French population to be ~4 μg/day [23].

Different lots of food were found to have different chromium concentrations [2, 7, 21, 22], and Anderson and colleagues concluded that "much chromium in foods may originate from external sources during growing, processing, preparation, fortification, and handling." Only a relatively small number of foods have been analyzed for chromium; the variability between different lots and different preparation techniques means that database values for chromium, even if they exist, are unlikely to provide an accurate assessment of a person's actual chromium intake.

## REPORTED CHROMIUM INTAKES OF EXCLUSIVELY BREAST-FED INFANTS

Chromium concentrations in breast milk were examined in 17 apparently healthy exclusively breast-feeding women at 60 days postpartum using a gas chromatograph-mass spectrophotometer with $^{50}Cr$ as an internal standard [24]. The mass ratio of $^{50}Cr$ to $^{52}Cr$ isotope was measured and the natural chromium in the breast milk samples was calculated. Over the three-day collection period the mean value was $3.43 + 0.39$ nmol Cr/L. Breast milk chromium did not increase with dietary chromium intake ($r = 0.03$). Based on an average breast milk consumption of 780 mL/day at 2 months postpartum [25], babies would have received 0.14 μg Cr/day. (This was in contrast to the previous ESADDI of 10–40 μg/day for infants aged <6 months.)

In a follow-up study, breast milk concentrations of six lactating women 1–2 months postpartum were measured. The mean chromium intake of their infants was 0.13 μg/day. Supplementing these women with a 400 μg dose of tracer $^{53}Cr$ for 3 days raised the $^{53}Cr$ concentrations in the serum but did not significantly increase $^{53}Cr$ or total chromium in breast milk [26].

Bioavailability of chromium from breast milk and formulas have not been evaluated in humans. However, in a study in rat pups using formulas or human milk extrinsically labeled with $^{51}Cr$, there were no significant differences in uptake or retention of $^{51}Cr$ in blood, urine, or organs at 6 hours after feeding suggesting similar bioavailability of these sources to the rat [27].

Based on the studies showing that 1–2-month-old infants received approximately 0.13 μg Cr/day [24, 26] and earlier studies suggesting breast milk chromium intakes of ~0.20 μg/day for infants [28, 29], the AI for infants 0–6 months was set at 0.2 μg/day.

## REPORTED CHROMIUM INTAKES FOR OLDER INFANTS, CHILDREN, AND ADOLESCENTS

There were no studies available that reported chromium intakes for older infants, children, or adolescents. For infants 7–12 months the AI was based on a combination of the chromium provided by human milk and by the usual intake of complementary foods. The average energy intake of infants in this age group is approximately 845 kcal and the mean volume of milk consumed by infants of this age is 0.6 L [25]. If human milk provides 750 kcal/L, the infant would get ~450 kcal from milk based on consuming 0.6 L. If the infant needs 845 kcal, 395 kcal would need to be provided by complementary weaning foods. Based on 13.4 μg Cr/1000 kcal, this 395 kcal would provide ~5.3 μg/day in addition to the ~0.2 μg/day provided by milk for an AI of 5.5 μg Cr/day.

Because no data were available on chromium intakes of children and adolescents aged 1–18 years, AIs for these age groups were extrapolated from adults, aged 19–30 years, by multiplying by a factor. The factor was $(\text{weight}_{\text{child}}/\text{weight}_{\text{adult}})^{0.75} \times (1 + \text{growth factor})$. The growth factor was 0.3 for children aged 1–3 years, 0.15 for males until 18 years of age and 0.15 for females until 14 years of age [1].

## REPORTED DIETARY CHROMIUM INTAKES OF ADULTS

Anderson and Kozlovsky [30] determined dietary chromium in self-selected diets for 10 males and 22 females. Each subject collected duplicate food and beverage composites for a 7-day period. Diets were collected in polyethylene containers and homogenized in a Waring blender with a low-chromium steel blade to minimize contamination. Samples were analyzed by graphite furnace atomic absorption spectroscopy. Bovine liver standard reference material, a low-chromium rat diet, and a composite human diet sample were also analyzed for quality control. The majority of diets contained between 10 and 40 μg Cr/day. Mean daily Cr intake for the 10 male subjects was $33 \pm 3$ μg/day (range 13–89 μg) and mean intake for the 22 women was $25 \pm 1$ μg/day (range 8–72 μg). Mean chromium intake for the males was 14 μg/1000 kcal and for females was 16 μg/1000 kcal. For all subjects together, the mean intake was $28 \pm 1$ μg/day or 15 μg/1000 kcal [30]. In another study, freely chosen diets from eleven women and eight men were collected using the duplicate plate method. Mean chromium concentration per 1000 kcal for men was $18.6 \pm 2.8$ μg and for women was $12.5 \pm 0.8$ μg. Mean chromium concentration for all subjects was $15 \pm 1.4$ μg/1000 kcal [22]. Also, nutritionists designed 22 well-balanced daily diets which were analyzed for trace mineral concentrations. Chromium concentration of these diets ranged from 8.4 to 23.7 μg/1000 kcal with a mean ± SEM (standard error of the mean) of $13.4 \pm 1.1$ μg/1000 kcal [22].

The percent of dietary Cr absorbed (estimated from urinary excretion of Cr) was inversely related to the total amount of Cr in the diet. When dietary Cr was 40 μg/day, only 0.4–0.5% of the dietary Cr was absorbed; however, when the intake was very low, such as 10 μg/day, approximately 2% of the Cr was absorbed [30]. Increased efficiency of absorption when chromium intakes are very low would be an efficient way to maintain chromium status and would be consistent with the absorptive behavior of several other trace elements.

Because no method existed for adjusting for underreporting of intake, the highest energy intake values within the adult age groups were used for each estimate [10]. This was 2800 kcal/day for men and 1850 kcal/day for women. Utilizing the 13.4 μg/day figure, chromium needs were estimated and rounded to establish the AI for adults.

## REPORTED CHROMIUM INTAKES OF THE ELDERLY

Chromium needs of elderly persons have been investigated in two studies. In one of these, dietary intake and excretion of 22 apparently healthy men and women aged 70–85 were measured for a 5-day period using duplicate diet techniques [31]. Chromium was analyzed by graphite furnace atomic absorption spectroscopy and National Bureau of Standards reference materials were monitored for quality control. Mean daily chromium intake for men was 29.8 μg and for women was 20.1 μg. Men were not statistically significantly different from women. The overall average intake was 24.5 μg/day or 12.8 μg/1000 kcal and the overall mean retention of chromium was 0.2 μg/day. The authors reported that 16 of the 22 subjects were in equilibrium for chromium. Three more were in positive balance. Two subjects were in slight negative balance and one was in severe negative balance. The subject in severe negative balance consumed a very

high fiber diet suggesting the need for further study of the effects of high fiber diets on chromium absorption.

A chromium balance study was conducted for 12 days in a metabolic unit with two ambulatory adult males, aged 62 and 66 years. Each man had received a constant metabolic diet containing 15.3 μg Cr/1000 kcal for the previous 3 months and this diet was continued through the study. Urinary chromium was 0.30 and 0.28 μg/day respectively, or 0.8% of intake. Apparent net retention of chromium was positive, at 0.6 and 0.2 μg/day respectively, indicating equilibrium [32].

Median energy intakes for men and women of 50–70 years of age are 2100 and 1500 kcal/day, respectively [10]. Intakes for men and women older than 70 years of age are 1700 and 1300 kcal/day, respectively [10]. The highest energy value for the ages above 50 was used as a basis for the chromium AI. Prevalence of glucose intolerance increases with aging [33] and a number of studies suggest that chromium needs of the elderly might be higher than those of young people. Bunker [31] found the most negative chromium balance in a person consuming a high fiber diet. Several medications may interfere with chromium absorption [34, 35], and chromium in hair seems to decrease with age [36].

## REPORTED CHROMIUM INTAKES DURING PREGNANCY AND LACTATION

Chromium may be depleted during pregnancy and particularly with multiple pregnancies [37]. Tissue chromium analysis conducted by Schroeder and colleagues [38] showed a decrease in tissue chromium concentrations with age in comparison to the concentrations in the fetus. Because of limitations to analytical techniques in the 1960s, there is a problem with the absolute chromium values, but the age distribution patterns are interesting. However, because of lack of data, the AI was determined by extrapolating from adolescent girls and adult women. The median weight gain during pregnancies with good outcomes was determined to be 16 kg [39], and this additional weight was added for extrapolation. Depending on the age of the woman, the AI for pregnancy was set at 29 or 30 μg/day.

The AI for lactation is based on the amount of chromium needed to replace chromium secreted in human milk plus the AI for women. To calculate an AI for chromium during lactation, if it is assumed that 1% of chromium is absorbed and 0.2 μg/day is secreted in human milk, an extra 20 μg/day of chromium is needed. The AI is similar to values observed for 17 lactating women who were 60 days postpartum. Mean daily energy intake of the lactating women was $2180 \pm 77$ kcal over the 3-day collection period. Daily chromium intake varied little and the mean dietary intake was $41 \pm 4$ μg/day [24].

## RESPONSES OF SUBJECTS TO CONTROLLED INTAKES OF CHROMIUM

There has been one controlled study where people consumed a low-chromium diet [24]. All 17 subjects (11 females and 6 males) consumed breakfast and dinner at the Beltsville

Human Nutrition Diet Facility and lunches and weekend meals were packed for each subject. All participants consumed low-chromium diets (5 μg/1000 kcal) for 14 weeks. Half of the subjects were supplemented with chromium for 4 weeks beginning at week 5 and half began the 4 weeks of chromium supplementation at week 10 in a crossover-type design. Urinary chromium tended to decrease with consumption of this very low-chromium diet. The mean urinary chromium was $4.56 \pm 1.35$ nmol/day at baseline when participants had been consuming self-selected diets and was $2.29 \pm 0.37$ nmol after consuming the low-chromium diet for 4 weeks. Eight of the subjects received chromium supplementation beginning at week 9 (i.e., in the second half of the crossover trial). In those subjects, there was a significant increase in glucose 90 minutes after a glucose load by week 9 when compared to week 0. Glucose concentrations were not affected in these subjects by 4 weeks of the low-chromium diet and fasting glucose also was not affected by 9 weeks of consuming 5 μg Cr/1000 kcal [1].

## EFFECTS OF VARIOUS DIETARY COMPONENTS ON CHROMIUM ABSORPTION AND EXCRETION

Apparently, a number of dietary components affect the absorption of chromium. Effects of simple versus complex dietary carbohydrate on urinary chromium excretion were investigated [40]. Thirty-seven healthy men and women volunteered for the 18-week study in which they ate breakfast and dinner in the research kitchen facility at the Beltsville Human Nutrition Research Center. Lunches and weekend meals also were provided "to go". The reference diet was designed by nutritionists to meet known nutrient needs. This reference diet consumed for 12 weeks contained 35% of total kilocalories as complex carbohydrate and 15% of kilocalories as simple sugars. A "simple sugar diet" with ~35% of kilocalories as simple sugar and 15% of kilocalories as complex carbohydrate was fed for 6 weeks. The chromium content of the reference diet contained $16.0 \pm 1.2$ μg Cr/1000 kcal and of the simple sugar diet was $15.7 \pm 0.8$ μg Cr/1000 kcal. Urinary chromium excretion was significantly higher during the simple sugar diet period than after consuming the reference diet. Furthermore, 27 of the subjects increased urinary chromium excretion in the simple sugar period while 10 subjects excreted more chromium with the reference diet. There was no gender difference in response. Overall, the proportion of dietary carbohydrate consumed as simple sugars seemed to lead to increased urinary chromium losses [40].

Ascorbic acid seems to enhance chromium absorption in rats and in human beings. In rats dosed concurrently with $^{51}CrCl_3$ and ascorbate, total urinary $^{51}Cr$ was increased without decreasing $^{51}Cr$ in the tissues suggesting greater absorption of $^{51}Cr$ [41]. Offenbacher and colleagues gave three women 1 mg $Cr^{3+}$ with or without 100 mg ascorbic acid on different days. For each woman, plasma chromium was higher when chromium was consumed with ascorbic acid than when consumed without ascorbic acid [42].

High levels of phytate impaired chromium absorption in rats [43], but lower levels did not seem to be detrimental [44, 45]. The question of effects of dietary fiber on chromium absorption needs further investigation. The one elderly subject in severely negative chromium balance in the study conducted by Bunker and colleagues [31] consumed a high fiber diet.

## EFFECTS OF METABOLIC STATES ON CHROMIUM NEEDS

Public interest is high in the potential for use of chromium as a supplement to assist in control of type II diabetes. There were no large-scale trials in the United States at the time the AI was established. Furthermore, the focus of the DRI is on recommendations for healthy people in the United States and Canada [1].

Chromium did relieve diabetic signs in a patient receiving TPN [46] and control of steroid-induced diabetes was improved by chromium supplementation [47]. In China, 180 subjects with type II diabetes were supplemented with placebo or with 200 or 1000 μg chromium picolinate per day for 4 months. After 2 months, fasting and 2-hour insulin concentrations and glycosylated hemoglobin were decreased significantly. Unfortunately, there is no information available on the dietary chromium intake of these patients [48].

## RESEARCH NEEDS

In order to determine an EAR, rigorously controlled studies are needed with intakes from $<5$ to 15–25 μg Cr/1000 kcal. A clinical indicator of marginal chromium status would greatly facilitate the research process as well as the evaluation of possible benefits of chromium supplementation for impaired glucose tolerance. Moreover, adverse effects of self-supplementation with chromium need to be systematically monitored; at the time the AI for chromium was established, there were few indications that elevated intakes of $Cr^{3+}$ were detrimental to human health.

## REFERENCES

[1] National Research Council (2002) *Dietary Reference Intakes for vitamin A, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A report of the Panel on Micronutrients, Subcommittee on Upper Reference Levels of Nutrients and of Interpretations and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes*. National Academy of Sciences, Washington, D. C., pp. 197–223.

[2] Anderson, R. A., Polansky, M. M., Bryden, N. A., and Canary, J. J. (1991) Supplemental chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am. J. Clin. Nutr. 54*, 909–916.

[3] National Research Council (1980) *Recommended Dietary Allowances, 9th Ed. Report of the Committee on Dietary Allowances, Division of Biological Sciences, Assembly of Life Science, Food and Nutrition Board, Commission on Life Science, National Research Council*. National Academy Press, Washington, D. C.

[4] National Research Council (1989) *Recommended Dietary Allowances, 10th Ed. Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Commission on Life Science, National Research Council*. National Academy Press, Washington, D. C.

[5] Veillon, C. (1986) Trace element analysis of biological samples. *Anal. Chem. 58*, 851A–858A.

[6] Veillon, C., and Patterson, K. Y. (1999) Analytical issues in nutraceutical chromium research. *J. Trace Elem. Exp. Med. 12*, 99–109.

[7] Kumpulainen, J. T. (1992) Chromium content of foods and diets. *Biol. Trace Elem. Res. 32*, 9–18.

[8] Guthrie, B. E., Wolf, W. R., and Veillon, C. (1978) Background correction and related problems in the determination of chromium in urine by graphite furnace atomic absorption spectrometry. *Anal. Chem. 50*, 1900–1902.

[9] Doisy, R. J., Streeten, D. H. P., Souma, M. L., Kalafer, M. E., Rekant, S. I., and Dalakos, T. G. (1971) "Metabolism of $^{51}$chromium in human subjects normal, elderly, and diabetic subjects" in *Newer Trace Elements in Nutrition* (Mertz, W., and Cornatzer, W. E., Eds.) pp 155–168, Marcel Dekker, Inc., New York.

[10] Briefel, R. R., McDowell, M. A., Alaimo, K., Caughman, C. R., Bischof, A. L., Carroll, M. D., and Johnson, C. L. (1995) Total energy intake of the US population: the third National Health and Nutrition Examination Survey, 1988–1991. *Am. J. Clin. Nutr. 62*, 1072S–1080S.

[11] Mertz, W. (1969) Chromium: occurrence and function in biological systems. *Physiol. Rev. 49*, 165–239.

[12] Schwarz, K., and Mertz, W. (1957) A glucose tolerance factor and its differentiation from factor 3. *Arch. Biochem. Biophys. 72*, 515–518.

[13] Jeejeebhoy, K. N., Chu, R. C., Marliss, E. B., Greenberg, G. R., and Bruce-Robertson, A. (1977) Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am. J. Clin. Nutr. 30*, 531–538.

[14] Anderson, R. A. (1987) "Chromium" in *Trace Elements in Human and Animal Nutrition* (Mertz, W., Ed.) pp 225–244, Academic Press, Inc., New York.

[15] Offenbacher, E. G., Pi-Sunyer, F. X., and Stoecker, B. J. (1995) "Chromium" in *Handbook of Nutritionally Essential Mineral Elements* (O'Dell, B. L., and Sunde, R. A., Eds.) pp 389–411, Marcel Dekker, Inc., New York.

[16] Stoecker, B. J. (2004) "Chromium" in *Elements and their Compounds in the Environment* (Merian, E., Anke, M., Ihnat, M., and Stoeppler, M., Eds.) pp 709–729, Wiley-VCH Verlag GmbH & Co., Weinheim.

[17] Vincent, J. B. (2000) The biochemistry of chromium. *J. Nutr. 130*, 715–718.

[18] Veillon, C. (1989) Analytical chemistry of chromium. *Sci. Total Envir. 86*, 65–68.

[19] Cary, E. E., and Kubota, J. (1990) Chromium concentration in plants: Effects of soil concentration and tissue contamination by soil. *J. Agric. Food Chem. 38*, 108–114.

[20] Offenbacher, E. G., and Pi-Sunyer, F. X. (1983) Temperature and pH effects on the release of chromium from stainless steel into water and fruit juices. *J. Agric. Food Chem. 31*, 89–92.

[21] Anderson, R. A., Bryden, N. A., and Polansky, M. M. (1988) Chromium content of selected breakfast cereals. *J. Food Comp. Anal. 1*, 303–308.

[22] Anderson, R. A., Bryden, N. A., and Polansky, M. M. (1992) Dietary chromium intake: Freely chosen diets, institutional diets, and individual foods. *Biol. Trace Elem. Res. 32*, 117–121.

[23] Cabrera-Vique, C., Teissedre, P. L., Cabanis, M. T., and Cabanis, J. C. (1997) Determination and levels of chromium in French wine and grapes by graphite furnace atomic absorption spectroscopy. *J. Agric. Food Chem. 45*, 1808–1811.

[24] Anderson, R. A., Bryden, N. A., Patterson, K. Y., Veillon, C., Andon, M. B., and Moser-Veillon, P. B. (1993) Breast milk chromium and its association with chromium intake, chromium excretion, and serum chromium. *Am. J. Clin. Nutr. 57*, 519–523.

[25] Heinig, M. J., Nommsen, L. A., Peerson, J. M., Lonnerdal, B., and Dewey, K. G. (1993) Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: the DARLING Study. *Am. J. Clin. Nutr. 58*, 152–161.

[26] Mohamedshah, F. Y., Moser-Veillon, P. B., Yamini, S., Douglass, L. W., Anderson, R. A., and Veillon, C. (1998) Distribution of a stable isotope of chromium ($^{53}$Cr) in serum, urine, and breast milk in lactating women. *Am. J. Clin. Nutr. 67*, 1250–1255.
[27] Payne, D. L., Adeleye, B., Hunt, D. J., and Stoecker, B. J. (1996) Chromium bioavailability from infant formulas and human milk in rats. *Biol. Trace Elem. Res. 53*, 1–6.
[28] Casey, C. E., and Hambidge, K. M. (1984) Chromium in human milk from American mothers. *Br. J. Nutr. 52*, 73–77.
[29] Casey, C. E., Hambidge, K. M., and Neville, M. C. (1985) Studies in human lactation: zinc, copper, manganese and chromium in human milk in the first month of lactation. *Am. J. Clin. Nutr. 41*, 1193–1200.
[30] Anderson, R. A., and Kozlovsky, A. S. (1985) Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr. 41*, 1177–1183.
[31] Bunker, V. W., Lawson, M. S., Delves, H. T., and Clayton, B. (1984) The uptake and excretion of chromium by the elderly. *Am. J. Clin. Nutr. 39*, 797–802.
[32] Offenbacher, E., Spencer, H., Dowling, H. J., and Pi-Sunyer, F. X. (1986) Metabolic chromium balances in man. *Am. J. Clin. Nutr. 44*, 77–82.
[33] Harris, M. I., Flegal, K. M., Cowie, C. C., Eberhardt, M. S., Goldstein, D. E., Little, R. R., Wiedmeyer, H. M., and Byrd-Holt, D. D. (1998) Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. The Third National Health and Nutrition Examination Survey, 1988–1994. *Diabetes Care 21*, 518–524.
[34] Kamath, S. M., Stoecker, B. J., Davis-Whitenack, M. L., Smith, M. M., Adeleye, B. O., and Sangiah, S. (1997) Absorption, retention and urinary excretion of chromium-51 in rats pretreated with indomethacin and dosed with dimethylprostaglandin $E_2$, misoprostol, or prostacyclin. *J. Nutr. 127*, 478–482.
[35] Martinez, O. B., Macdonald, A. C., Gibson, R. S., and Bourn, D. (1985) Dietary chromium and effect of chromium supplementation on glucose tolerance of elderly Canadian women. *Nutr. Res. 5*, 609–620.
[36] Davies, S., Howard, J. M., Hunnisett, A., and Howard, M. (1997) Age-related decreases in chromium levels in 51,665 hair, sweat, and serum samples from 40,872 patients – Implications for the prevention of cardiovascular disease and type II diabetes mellitus. *Metabolism 46*, 469–473.
[37] Mahalko, J. R., and Bennion, M. (1976) The effect of parity and time between pregnancies on maternal hair chromium concentration. *Am. J. Clin. Nutr. 29*, 1069–1072.
[38] Schroeder, H. A., Balassa, J. J., and Tipton, I. H. (1962) Abnormal trace metals in man–chromium. *J. Chron. Dis. 15*, 941–964.
[39] Carmichael, S., Abrams, B., and Selvin, S. (1997) The pattern of maternal weight gain in women with good pregnancy outcomes. *Am. J. Pub. Hlth. 87*, 1984–1988.
[40] Kozlovsky, A. S., Moser, P. B., Reiser, S., and Anderson, R. A. (1986) Effects of diets high in simple sugars on urinary chromium losses. *Metabolism 35*, 515–518.
[41] Seaborn, C. D., and Stoecker, B. J. (1990) Effects of antacid or ascorbic acid on tissue accumulation and urinary excretion of 51chromium. *Nutr. Res. 10*, 1401–1407.
[42] Offenbacher, E. G. (1994) Promotion of chromium absorption by ascorbic acid. *Trace Elem. Elect. 11*, 178–181.
[43] Chen, N. S. C., Tsai, A., and Dyer, I. A. (1973) Effect of chelating agents on chromium absorption in rats. *J. Nutr. 103*, 1182–1186.
[44] Keim, K. S., Stoecker, B. J., and Henley, S. (1987) Chromium status of the rat as affected by phytate. *Nutr. Res. 7*, 253–263.
[45] Keim, K. S., Holloway, C. L., and Hegsted, M. (1987) Absorption of chromium as affected by wheat bran. *Cereal Chem. 64*, 352–355.
[46] Freund, H., Atamian, S., and Fischer, J. E. (1979) Chromium deficiency during total parenteral nutrition. *J. Am. Med. Assoc. 241*, 496–498.

[47] Ravina, A., Slezak, L., Mirsky, N., and Anderson, R. A. (1999) Control of steroid-induced diabetes with supplemental chromium. *J. Trace Elem. Exp. Med. 12*, 375–378.

[48] Anderson, R. A., Cheng, N. Z., Bryden, N. A., Polansky, M. M., Cheng, N. P., Chi, J. M., and Feng, J. G. (1997) Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.

This page intentionally left blank

*Chapter 3*

# Multiple hypotheses for chromium(III) biochemistry: Why the essentiality of chromium(III) is still questioned

**Diane M. Stearns**

Department of Chemistry and Biochemistry, Northern Arizona University, Flagstaff, Arizona 86011–5698

## INTRODUCTION

Trivalent chromium ($Cr^{3+}$) has generally been accepted to be a trace essential metal since Schwartz and Mertz first concluded that addition of $Cr^{3+}$ salts to a rat diet increased blood glucose removal [1]. It is currently accepted by many to maintain normal blood sugar levels, regulate proper carbohydrate and lipid metabolism, and to enhance insulin signaling, as discussed in other chapters in this text. The strongest advocates for this paradigm have included those involved with the chromium supplement industry [2–9], which has been quite lucrative since the US sales of chromium supplements have been reported to be second only to those of calcium [10].

The field of $Cr^{3+}$ biochemistry has been carried out in the reverse of normal hypothesis-driven science because it was first labeled as essential, then the past 50 years have been spent trying to find proof, with well-meaning investigators struggling to come up with interpretations of their data that did not show what was expected. This author offers the idea of chromium essentiality as one more example of a field of research for which "a single hypothesis is all too often championed at the expense of a reasoned approach that requires entertaining a series of complex arguments until one or more can be discarded on the basis of experimental data [11]." The essentiality of chromium is not the first, nor likely the last field of science to fall into this trap. Thomas Chrowder Chamberlin (1843–1928), former president of the University of Wisconsin and the American Association for the Advancement of Science, eloquently warned against this type of investigation in 1890 when he called for using the "Method of Multiple Working Hypotheses" in which incorrect theories can be avoided if scientists allow all possible interpretations of data while trying to explain an observed phenomenon [12]:

> The moment one has offered an original explanation for a phenomenon which seems satisfactory, that moment affection for his intellectual child springs into existence; and as the explanation grows into a definite theory, his parental affections cluster

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

> about his intellectual offspring, and it grows more and more dear to him, so that, while he holds it seemingly tentative, it is still lovingly tentative, and not impartially tentative. So soon as this parental affection takes possession of the mind, there is a rapid passage to the adoption of the theory. There is an unconscious selection and magnifying of phenomena that fall into harmony with the theory and support it, and an unconscious neglect of those that fail of coincidence. The mind lingers with pleasure upon the facts that fall happily into the embrace of the theory, and feels a natural coldness toward those that seem refactory. . . There springs up, also, an unconscious pressing of the theory to make it fit the facts, and a pressing of the facts to fit the theory. When these biasing tendencies set in, the mind rapidly degenerates into the partiality of paternalism. The search for facts, the observation of phenomenon and their interpretation, are all dominated by affection for the favored theory until it appears to its author or its advocate to have been overwhelmingly established.

The aim of this review is thus to introduce the "Method of Multiple Working Hypotheses" to the field of chromium biochemistry by presenting the major possible hypotheses that may or may not explain the data surrounding the biochemistry of chromium, with a summary of the data that support or weaken them. Five years ago, this author first surveyed the literature in an attempt to demonstrate the lack of consistent evidence underlying the major assumptions behind the function of $Cr^{3+}$ as an essential metal [13]. Specifically, (1) that cases of $Cr^{3+}$ supplementation in total parenteral nutrition showed no consistent patterns; (2) that no Cr-containing glucose tolerance factor has been reproducibly isolated and characterized; (3) that the existence of a biologically active low molecular weight chromium (LMWCr)–binding protein (chromodulin) has not been distinguished from a phase II metabolism product of the intracellular reduction of $Cr^{6+}$; (4) that $Cr^{3+}$, as the final product in the biological reduction of $Cr^{6+}$, has not been ruled out in the overall pathway of $Cr^{6+}$-induced cancers; (5) that no consistent reproducible evidence exists for $Cr^{3+}$ deficiencies in humans or animals; and lastly (6) that the effect of $Cr^{3+}$ on iron metabolism has not been thoroughly investigated.

Five years later, we are no closer to proving if or how $Cr^{3+}$ is essential, which further supports the growing need to reconsider the chromium paradigm. Three major hypotheses have been proposed to explain how $Cr^{3+}$ functions in the body: the glucose tolerance factor, chromodulin, and interference in iron metabolism. Recent studies addressing these hypotheses will be presented. A fourth hypothesis is introduced here, that of chromium hormesis. But first, the stage may be set by considering the data that have not been consistent with $Cr^{3+}$ essentiality, specifically inconsistent observations of patients on total parenteral nutrition, lack of established deficiencies in humans, and studies showing no effects of $Cr^{3+}$ supplementation on study populations.

## DATA NOT CONSISTENT WITH CHROMIUM ESSENTIALITY

### Total parenteral nutrition

Evidence for the essentiality of chromium is purported to come from cases of long-term total parenteral nutrition. Five cases were reported between 1977 and 1998 in which addition of Cr to TPN was concluded to affect glucose intolerance and or neuropathy [14–18]. However, taken as a whole, these cases did not show a consistent relationship between observed symptoms and either length of time on TPN, baseline

or supplemented levels of Cr in TPN, or levels of Cr in serum before or after supplementation. No new cases of Cr deficiency as a result of TPN have been reported since the original five. However, one recent study of short-term Cr supplementation in five patients on TPN found two patients who showed "possible benefit" and three patients who showed little or no benefit after receiving chromic chloride at 10 μg Cr/day for 2 days, 50 μg Cr/day for 3 days, and 10 μg Cr/day for 3 days [19]. Discerning the function of Cr in all of these patients is undoubtedly complicated by the severe health problems that required them to be on TPN in the first place. Thus these patients are not an ideal group in which to investigate the essentiality and function of Cr; however, no alternate group of people has consistently and reproducibly shown evidence of Cr deficiency that can be alleviated by dietary supplementation.

**Dietary deficiencies**

A review of the daily adequate intake for chromium is covered elsewhere in this text. Three conclusions commonly stated as evidence of human dietary deficiencies are, in fact, misinterpretations of the literature. They have been reviewed previously [13], and may be summarized as (1) self-selected dietary intakes of $Cr^{3+}$ have been measured at 13–56 μg Cr/day [20–22], and have been defined as "inadequate" because older values of the estimated safe and adequate daily dietary intake (50–200 μg Cr/day) were based on measurements made with poor analytical techniques and were inaccurately high; (2) tissue levels of Cr have been asserted to decrease with age [23], but this claim has not been consistently supported by independent studies [24–27]; and (3) increased uptake of $Cr^{3+}$ results in decreased absorption [21], but this has yet to be demonstrated within individual subjects. There is currently no reproducible direct evidence of chromium dietary deficiencies in human subjects.

**Supplementation**

In spite of a lack of evidence of wide-spread Cr deficiency in humans, $Cr^{3+}$ dietary supplementation is recommended for regulation of blood glucose levels in people with type II diabetes. A review of studies of the effects of $Cr^{3+}$ supplementation in humans is addressed elsewhere in this text and is beyond the scope of this chapter, except to note that a recent meta-analysis of data from randomized clinic trials has shown that chromium supplementation had no effect on glucose or insulin concentrations in nondiabetic subjects, and had inconclusive effects on diabetic subjects [28].

In the absence of a defined subpopulation that consistently and reproducibly demonstrates a clear chromium deficiency, it becomes imperative to define the essentiality of chromium in terms of its molecular mechanism. The two most popular candidates for the biologically active form of $Cr^{3+}$ are the Cr-containing glucose tolerance factor and the LMWCr–binding protein; but unfortunately, investigations of these two species have also been fraught with problems of consistency and reproducibility.

## THE CHROMIUM GLUCOSE TOLERANCE FACTOR

The label of $Cr^{3+}$ essentiality was bestowed ahead of direct scientific evidence showing that $Cr^{3+}$ was present in any enzyme or cofactor. A chromium-containing glucose tolerance factor was originally introduced as the biologically active form of $Cr^{3+}$ by

Mertz and coworkers [1, 29–31], and this highly cited first model for the biological function of $Cr^{3+}$, namely a $Cr^{3+}$ complex of niacin, glycine, cysteine, and glutamic acid that formed a complex with insulin and the insulin receptor [30], was complete speculation at the time it was published and has not been substantiated. Several reviews, including other chapters in this text, describe isolation, purification, and testing of various forms of glucose tolerance factor [13, 32–34] and will not be repeated here. More recent attention has been focused on the LMWCr-binding protein, specifically in terms of characterization of its structure and function.

## THE LMWCr-BINDING PROTEIN

### Structural characterization of LMWCr

An LMWCr-binding protein was first found in mouse liver after injection with hexavalent chromium ($Cr^{6+}$) as potassium dichromate [35] and was further characterized in dog liver [36]. Its biological relevance has not yet been unambiguously established. For example, studies in mice reported that less LMWCr was obtained after in vivo exposure of mice to $Cr^{6+}$ than after soaking of tissue with $Cr^{6+}$ ex vivo, and that in vivo exposure to $Cr^{6+}$ did not increase expression of apoprotein [37]. Nevertheless, similar substances were also found in rabbit liver [38] and bovine colostrum [39], and the apoprotein was confirmed to bind $Cr^{3+}$ in vitro. Vincent and coworkers subsequently adapted the $Cr^{6+}$ soaking procedure to isolate LMWCr from bovine liver. A sample of 30 mg of LMWCr was obtained from 5 to 6 kg of diced bovine liver tissue that had been loaded ex vivo with $Cr^{6+}$ [40], with a lower yield obtained with $Cr^{3+}$ instead of $Cr^{6+}$. Differences among results of ex vivo loading with $Cr^{6+}$ and in vivo incorporation of $Cr^{3+}$ from either $Cr^{3+}$ or $Cr^{6+}$ suggest the possibility that the observed products may be artifacts, and not the result of essential biological processes.

An initial characterization of LMWCr has been provided. The $Cr^{3+}$:amino termini ratio was reported to be 3.5 : 1; however, the peptide lost $Cr^{3+}$ over time, presumably by hydrolysis [40]. The peptide portion of the 1500 molecular weight complex consisted of aspartate, glutamate, glycine, and cysteine residues at a ratio of 2.15 : 4.47 : 2.47 : 2.35, which is similar but not identical to LMWCr from other sources [36, 38, 39]. Attempts to sequence the apoprotein have been unsuccessful so far.

One of the difficulties of characterizing this protein is the general lack of a good spectroscopic handle for $Cr^{3+}$. The UV/visible spectrum for LMWCr was similar to those of known $Cr^{3+}$ oxotrimers, although the peptide was inferred to contain a tetranuclear core, but the $^1H$ NMR and EPR spectra of LMWCr were relatively featureless [40]. Also, perhaps more importantly, no analysis was provided to verify that all the Cr in the preparation was reduced from $Cr^{6+}$ to $Cr^{3+}$, which may have implications for its inferred biological activity.

Several other issues regarding the characterization of LMWCr have also been raised in a recent review [41]. Specifically, the existence of a polynuclear assembly has not been directly confirmed, elemental analysis and X-ray crystallographic data to confirm the presence of four Cr atoms per protein are currently lacking, the similarities of spectral data to those of $Cr^{3+}$ GSSG complexes (where GSSG is the disulfide-bridged oxidized glutathione tripeptide dimer) are striking (vide infra), there are limitations to

determining molecular mass by gel filtration that could be overcome by mass spectrometric techniques, indirect measurement of the Cr-protein binding constant has inherent errors, and the fact that higher yields of LMWCr are obtained from $Cr^{6+}$ rather than $Cr^{3+}$ raises questions as to the biological relevance of LMWCr (vide infra).

An alternate hypothesis that may explain the data surrounding LMWCr is that it represents nonspecific protein or amino acid binding of $Cr^{3+}$ [13]. Much has been published on the characterization and function of LMWCr in terms of its being the essential form of $Cr^{3+}$; however, it has all come from a single laboratory [50]. A recent study by Gaggelli and coworkers described attempts to isolate LMWCr following published methods [40], but the characterization of the product that was obtained did not match published data in terms of its UV/visible, mass spectrometry, or $^{1}H$ NMR spectroscopy spectra [42]. This difference in isolated product is further supported by the original finding that the LMWCr isolated from bovine liver by Vincent and coworkers using Yamamoto's protocol [40] did not match what Yamamoto originally found in rabbit or dog liver, or bovine colostrum [36, 38, 39]. Furthermore, Gaggelli and coworkers concluded that simple mixtures of $Cr^{6+}$ with glutathione, aspartate, and glutamate yielded products that closely modeled the mixture of species that was obtained from homogenized liver that had been treated with $Cr^{6+}$ [42]. These studies demonstrate the need for more complete direct characterization of the $Cr^{3+}$ coordination environment in both Cr-containing proteins and $Cr^{3+}$ compounds with biologically relevant ligands.

A more thorough analysis of the proteins that bind to $Cr^{3+}$ in vivo has recently been provided by stable isotope tracer studies using $^{50}Cr^{3+}$ as $^{50}Cr_2O_3$ [43, 44]. In the first study, in which normal and diabetic rats were injected with $^{50}Cr_2O_3$, the majority of $Cr^{3+}$ was found to be distributed in a high molecular weight fraction with a UV absorbance of 280 nm, and a minor amount of $Cr^{3+}$ was present in a low molecular weight fraction with a UV absorbance of 260 nm [43]. In both animal groups, almost 60% of the total $Cr^{3+}$ was found in the high molecular weight fraction in the rat liver, and approximately 90% of total Cr was found in the high molecular weight fraction in serum. However, LMWCr was the only form of $Cr^{3+}$ observed in rat urine. The LMWCr also represented 27–37% of total Cr in rat liver and 8–10% of total Cr in serum, with the remaining $Cr^{3+}$ inferred to exist as inorganic salts or other small molecules. Thus these data were consistent with Yamamoto and coworkers' conclusion that LMWCr was the form of $Cr^{3+}$ excreted in animals, but was in contrast to their conclusion that LMWCr was the predominant form of Cr in animal liver [35, 38].

Further characterization of Cr-containing proteins through $^{50}Cr^{3+}$ isotope tracer studies examined subcellular distribution of $Cr^{3+}$ in rat liver and showed that the highest amount was present in nuclei, and amounts decreased in the order: nuclei > mitochondria > lysosome > microsome > cytosol [44]. Nine kinds of high molecular weight Cr-containing proteins were identified in rat liver, with masses ranging from 97 to 4 kDa. Nuclei contained the highest levels of $Cr^{3+}$, and within nuclei, 77% of the Cr-containing compounds had weights of approximately 9, 97, and 37 kDa. These data support the interpretation that $Cr^{3+}$ binding by protein is much less specific than previously proposed.

The relative distribution of $Cr^{3+}$ between high and low molecular weight proteins may also be influenced by the dose of Cr to which the animals are experimentally exposed. Comparison of the doses used by Yamamoto and coworkers [35–39], Vincent and coworkers [40], and Feng and coworkers [43, 44] suggests that higher doses of

$Cr^{6+}$ or $Cr^{3+}$ result in greater amounts of $LMWCr^{3+}$, while injections of Cr at more physiologically relevant levels favor binding by high molecular weight proteins [44]. This supports the original interpretation that LMWCr is involved in the "detoxification of excessive absorbed chromium "[35], and is less consistent with the assumption that LMWCr is the relevant form through which $Cr^{3+}$ carries out its role as an essential metal in humans.

While some investigators are seeking more direct characterization of LMWCr, others have attempted to compare possible sequences of the LMWCr peptide with other known proteins [45]. A bioinformatics search of protein databases looked for matches between all permutations for the 10 amino acids reported for the stoichiometry of LMWCr, E : G : C : D :: 4 : 2 : 2 : 2, and known proteins. Three exact matches were found: EDGEECDCGE, DGEECDCGEE, both found in ADAM 19 (A Disintegrin and Metalloproteinase domain 19 protein) from humans and mice, and CEGGCEEDDE, found in a protein kinase in rice. A search for the pentameric sequence E : G : C : D :: 2 : 1 : 1 : 1, which could correspond to a disulfide-bridged peptide, did find a match with –EECGD-, existing in residues 175–184 of the $\alpha$-subunit of the insulin receptor [45]. In the absence of an as of yet unsequenced or unidentified new protein, these results could be interpreted to suggest that LMWCr is not necessarily a fragment of the insulin binding receptor, but that $Cr^{3+}$ generally favors carboxylate-rich stretches of amino acid sequences which happen to be present in the insulin binding receptor and other proteins. Thus these results could be interpreted to support the "nonspecific binding hypothesis" for biological $Cr^{3+}$, but also may help to explain why it has been experimentally linked with the insulin-signaling pathway.

**Functional characterization of LMWCr**

Two hypotheses have been proposed to explain how $Cr^{3+}$ acts in the insulin-signaling pathway. The first hypothesis is that the LMWCr protein participates in an autoamplification system for insulin signaling. Yamamoto and coworkers originally reported that LMWCr isolated from several sources increased production of $^{14}CO_2$ from [U-$^{14}$C]glucose in the presence of insulin in isolated rat adipocytes [38, 39, 46] More recently, LMWCr has been reported to activate phosphotyrosine phosphatase activity in isolated adipocyte membranes [47] and to activate insulin receptor tyrosine kinase activity in the presence of insulin in rat adipocyte membrane fragments [48]. These experiments have served as a springboard for a proposed mechanism for Cr-activated insulin signaling in which insulin binds to its external alpha subunit of the insulin receptor on a relevant cell, $Cr^{3+}$ enters the insulin-dependent cell where it binds to apo-LMWCr, and the haloLMWCr binds to the intracellular beta subunit of the insulin receptor to amplify receptor kinase activity [49]. All of these steps remain to be experimentally verified directly; however, that has not stopped this mechanism from being propagated in subsequent reviews [50, 51], thus this scheme may be on its way to replacing the figure of GTF that served as the explanation for the biological function of $Cr^{3+}$ for 40 years until it was finally toppled by a lack of experimental support.

An alternate hypothesis has been put forth to explain the data leading to the proposed mechanisms of action of LMWCr which involves oxidation of $Cr^{3+}$ to $Cr^{6+}$ under physiological conditions. The core of LMWCr has been proposed to exist in a $\mu$-oxo trimer configuration [40, 52], modeled by the synthetic carboxylate bridged

μ-oxotrimers [53, 54]. Using the propionate oxotrimer as an example, Lay and coworkers have demonstrated that this $Cr^{3+}$ oxotrimer will undergo oxidation of $Cr^{6+}$ in the presence of hydrogen peroxide ($H_2O_2$) chlorite ($ClO^-$), glucose oxidase, and xanthine oxidase, and the resulting $Cr^{6+}$ was shown to inhibit protein tyrosine phosphatase (*Yersinia enterocolitica*) [55]. Those authors suggested that inhibition of phosphatases by $Cr^{5+}$ or $Cr^{6+}$ oxidation products could be responsible for chromium's insulin-mimetic effects.

Lastly, the question of specificity has not been fully addressed experimentally. The observation that a $Cr^{3+}$-containing LMWCr stimulated the tyrosine kinase activity of the insulin-activated insulin receptor does not in itself prove that it is essential, because other drugs will produce the same stimulation. For example, the anti-diabetic drug metformin (*N*, *N*′-dimethylbiguanide) was shown to stimulate insulin receptor tyrosine kinase activity and inhibit tyrosine phosphatases in the *Xenopus* oocyte [56], and to increase insulin-stimulated glucose transport in isolated rat adipocytes [57]. Another drug used to treat NIDDM, pioglitazone (5-[4-(2-(5-ethyl 12-pyridyl)ethoxy]- benzoyl]-2,4-thiazolidinedione) was reported to increase insulin sensitivity in Wistar fatty rats by activating insulin receptor kinases [58]. Zinc has broad insulinomimetic effects including inhibition of protein tyrosine phosphatases in rat glioma cells [59] and increase in glucose transport in rat adipocytes [60]. Divalent Mn was found to increase insulin-stimulated glucose transport [61] and oxidation [62, 63] in isolated rat adipocytes, and conversely, glucose transport and metabolism were decreased in adipocytes from Mn-deficient rats [64]. Cadmium increased insulin-stimulated glucose oxidation in rat adipocytes [63]. Chromium$^{6+}$ was found to be an insulin mimetic in rat adipocytes [65], and to increase tyrosine phosphorylation in human lung A549 cells [66] and rat hepatoma cells [67]. Thus standardization of tests and side-by-side comparisons of $Cr^{3+}$ and similar acting molecules may help to elucidate if and how $Cr^{3+}$ compounds are unique in terms of their involvement in insulin-related signaling pathways.

In summary, many issues need to be addressed before the biological role of $Cr^{3+}$ comes into focus. Isolation and characterization of biological $Cr^{3+}$ is not reproducible across laboratories, because $Cr^{3+}$ binds to multiple proteins in vivo. No characterization or activity studies have yet been carried out with the predominant forms of $Cr^{3+}$ formed in vivo, namely the high molecular weight Cr species, to determine if they have any level of biological activity. Oxidation of $Cr^{3+}$ to $Cr^{6+}$ in vivo can produce phosphatase inhibition. Stimulation of the kinase activity of the insulin-activated insulin receptor is also produced by several metals and non-essential drugs. Therefore, the model of biologically active $Cr^{3+}$ existing as "chromodulin" and acting in insulin-signaling autoamplification, as attractive as that theory may be, could at the present time be an oversimplification of the existing data. The alternate hypothesis, that the observed function of $Cr^{3+}$ is pharmacological rather than essential, has not been ruled out.

## RELATION OF CHROMIUM METABOLISM TO IRON METABOLISM

The original hypothesis that the effects of chromium on parameters related to diabetes may, in fact, be due to either influences of chromium on iron metabolism or influences of iron on chromium metabolism has not yet been fully explored [13]. A growing number of studies have reported a link between high iron levels and both type II and gestational

diabetes [68–71]. It has been well established that iron-binding proteins are involved in chromium binding, transport, and storage [44, 72–74]. Chromium blood plasma levels have been reported to be lower in diabetics than in non-diabetic controls [75–79]. Thus, decreased chromium stores in diabetics could be an indirect indication of increased iron stores, not necessarily a cause of diabetes. Also, positive effects attributed to chromium supplementation could be a result of lowered iron absorption rather than an improvement in a chromium deficiency. Further study of the link between chromium and iron metabolism is needed to support these hypotheses.

## HORMESIS

The concept of dose-response, that there is a proportional relationship between the amount of exposure to a chemical or physical agent and the degree of response in a given experimental system, is a central tenet of pharmacology and toxicology. However, the emerging theory of hormesis is at odds with this basic definition. Hormesis, derived from the Greek word *hormaein* (to urge on, excite), is the observation of a stimulatory or "beneficial" response elicited by a low dose range of a chemical, even if that chemical produces an inhibitory or adverse response at a higher dose range. Hormesis is manifested as a biphasic dose–response curve that consists of a low dose range that is stimulatory, a moderate dose range with little or no response, and a high dose range that is inhibitory or toxic. It is thought to be caused either by an overcompensation due to initial disruption of homeostasis, or by direct stimulation [80].

The concept of hormesis was first proposed by Southam and Erlich in the 1940s after observing that low doses of cedar bark extract stimulated fungal growth [81]. It has received an increasing amount of attention by the scientific community through the efforts of Calabrese and Baldwin who have exhaustively followed and cataloged the hormetic responses in microbial, plant, and animal models exposed to chemical and physical agents [82, 83] including ethanol [84], peptides [85], radiation [86], and metals [87].

Hormetic responses have been observed with several metals and these results may have relevance to the issue of chromium essentiality. One of the best examples is arsenic, an established human carcinogen. Cancer risks due to exposure to arsenic in drinking water have been reported to actually be lower in populations exposed to $\leq$60 ppb As relative to unexposed control populations [88], purportedly due to arsenic's protective effects against oxidative damage and DNA repair inhibition at low doses [89]. Not surprising, $As^{3+}$ has been proposed by some to be essential in humans and/or animals [90–92].

Another example with possible relevance to $Cr^{3+}$ is the observation that low levels of cadmium, mercury, and copper salts induced stress-dependent increases in glucose uptake in McCoy mouse cells that were protein kinase-C dependent, and decreases in mitochondrial respiration that were dependent on protein synthesis [93]. Enhanced glucose uptake has been one of the hallmark measurements interpreted as proof of $Cr^{3+}$ essentiality [94].

Other non-essential metals besides cadmium and mercury that have been shown to display hormesis include aluminum, gold, lead, lithium, platinum, tributyl tin, and uranium, as well as the inorganic species fluoride, hypochlorite ($OCl^-$), and iodine [87].

Less work has been carried out to evaluate possible hormetic effects of $Cr^{3+}$ or $Cr^{6+}$; however, some studies suggest that such effects may occur. Low doses of $Cr^{6+}$ as $CrO_3$ were found to enhance the fidelity of bacterial DNA repair by *Escherichia coli* DNA polymerase I, whereas higher doses inhibited fidelity [95]. Trivalent chromium as $CrCl_3$ was found to inhibit onion root growth and mitotic index during 72-hr exposures, but stimulated root growth during a 96-hr recovery period [96]. Exposure of mustard plants to low levels of $Cr^{6+}$ as $K_2Cr_2O_7$ (0.20 μM) produced a stimulation of biomass over 5 days of exposure that was lost at higher doses or longer treatment times [97]. Hexavalent chromium as $CrO_3$ or $K_2Cr_2O_7$ stimulated norepinephrine (NE) release from bovine adrenal medullary cells at doses $<10$ μM $Cr^{6+}$, but inhibited NE release at doses $>100$ μM, and chromic chloride was able to stimulate NE release in cells permeabilized with digitonin [98].

These initial data from in vitro, plants, and cell culture experiments suggest that hormetic effects may well be induced by $Cr^{3+}$. Therefore, attempts to rectify hormesis theory with respect to that of $Cr^{3+}$ essentiality will need to be made before the biochemistry of $Cr^{3+}$ can be fully understood. Just because a cell, animal, or human subject senses and responds to $Cr^{3+}$ does not in itself prove that $Cr^{3+}$ is essential.

## CONCLUSIONS

The purpose of the current review was to survey the field 5 years after an initial review [13] to analyze the extent to which we have come closer to proving if or how $Cr^{3+}$ is essential. During this time frame the existence of Cr-GTF has been shown to be even less likely, and the isolation of an LMWCr-binding protein has not been found to be reproducible. The role of $Cr^{3+}$ in iron metabolism has not been adequately investigated in the last 5 years, and so remains an open question. Finally, it is proposed that the general ability of $Cr^{3+}$ to display hormesis should be explored as a possible explanation for its observed biological activity.

The role of $Cr^{3+}$ in biology can only be properly explained by holding up all possible mechanisms of its activity for equal scrutiny, rather than interpreting data to fit a chosen theory. The benefits of this more objective type of endeavor have been summarized by Chamberlin [12]:

> Going with this effort there is a predisposition to balance all evidence fairly, and to accept that interpretation to which the weight of evidence inclines, not that which simply fits our working hypothesis or our dominant theory. The outcome, therefore, is better and truer observation and juster and more righteous interpretation.

## REFERENCES

[1] Schwartz, K., and Mertz, W. (1959) Chromium(III) and the glucose tolerance factor. *Arch. Biochem. Biophys. 85*, 292–295.
[2] Skogerson, L. E. (1982) US Patent No. 4,348,483. Method for the production of chromium yeast.
[3] Jensen, N. L. (1990) US Patent No. 4,923,855. Synthetic GTF chromium material and process therefore.

[4] Boynton, H., and Evans, G. W. (1992) US Patent No. 5,087,623. Chromium Picolinate Treatment.
[5] Wong, Y. (1996) US Patent No. 5,536,838. Method of synthesis of chromium amino acid nicotinate complex.
[6] Vincent, J. B., and Davis, C. M. (1999) US Patent No. 5,872,102. Method for isolation of bovine low-molecular weight Cr-binding substance and method of use of the same.
[7] Vincent, J. B., and Davis, C. M. (2001) US Patent No. 6,197,816. Use of triaqua-mu3-oxohexakis-mu-propionatotrichromium (1+), $[Cr_3O(O_2CCH_2CH_3)6(H_2O)_3]^+$, as a nutritional supplement or in treatment of medical conditions.
[8] McCarty, M. F. (2001) US Patent No. 6,329,361. High-dose chromic picolinate treatment of type II diabetes.
[9] Yu, K., Kou, F., and Wang, Z. (2003). US Patent No. 6,548,687. Chromium L-threonate, process for preparation of the same and their use.
[10] Nielsen, F. H. (1996) Controversial chromium: does the superstar mineral of the mountebanks receive appropriate attention from clinicians and nutritionists? *Nutr. Today 31*, 226–233.
[11] Prusiner, S. B. (1998) Prions. *Proc. Natl. Acad. Sci. USA 95*, 13363–13383.
[12] Chamberlin, T. C. (1890) The method of multiple working hypotheses. *Science [Old Series] 15*, 92–97. Reprinted in (1965) *Science 148*, 754–759.
[13] Stearns, D. M. (2000) Is chromium a trace essential metal? *Biofactors 11*, 149–162.
[14] Jeejeebhoy, K. N., Chu, R. C., Marliss, E. B., Greenberg, G. R., and Bruce-Robertson, A. (1977). Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am. J. Clin. Nutr. 30*, 531–538.
[15] Freund, H., Atamian, S., and Fischer, J. F. (1979) Chromium deficiency during total parenteral nutrition. *JAMA 241*, 496–498.
[16] Brown, R. O., Forloines-Lynn, S., Cross, R. E., and Heizer, W. D. (1986) Chromium deficiency after long-term total parenteral nutrition. *Dig. Dis. Sci. 31*, 661–664.
[17] Anonymous (1988) Is chromium essential for humans? *Nutr. Rev. 46*, 17–20.
[18] Tsuda, J., Yokoyama, Y., Morita, M., Nakazawa, Y., and Onishi, S. (1998) Selenium and chromium deficiency during long-term home total parenteral nutrition in chronic idiopathic intestinal pseudoobstruction. *Nutrition 14*, 291–295.
[19] Wongseelashote, O., Daly, M. A., and Frankel, E. H. (2004) High insulin requirement versus high chromium requirement in patients nourished with total parenteral nutrition. *Nutrition 20*, 318–320.
[20] Bunker, V. W., Lawson, M. S., Delves, H. T., and Clayton, B. E. (1984) The uptake and excretion of chromium by the elderly. *Am. J. Clin. Nutr. 39*, 797–802.
[21] Anderson, R. A., and Kozlovsky, A. S. (1985) Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr. 41*, 1177–1183.
[22] Offenbacher, E. G., Spencer, H., Dowling, H. J., and Pi-Sunyer, F. X. (1986) Metabolic chromium balances in men. *Am. J. Clin. Nutr. 44*, 77–82.
[23] Schroeder, H. A., Balassa, J. J., and Tipton, I. H. (1962) Abnormal trace metals in man—chromium. *J. Chronic. Dis. 15*, 941–964.
[24] Versiek, J. (1985) Trace elements in human body fluids and tissues. *CRC Crit. Rev. Clin. Sci. 22*, 97–184.
[25] Vuori E., and Kumpulainen, J. (1987) A new low level of chromium in human liver and spleen. *Trace Elem. Med. 4*, 88–91.
[26] Shah, B. G., and Belonje, B. (1990) Levels of magnesium, copper, chromium, manganese and cadmium in the livers of Canadians. *Trace Elem. Med. 7*, 11–18.
[27] Lyon, T. D. B., and Fell, G. S. (1992) Trace elements in autopsy tissue. *Food Chem. 43*, 299–306.

[28] Althuis, M. D., Jordan, N. E., Ludington, E. A., and Wittes, J. T. (2002) Glucose and insulin responses to dietary chromium supplements: a meta-analysis. *Am. J. Clin. Nutr. 76*, 148–155.
[29] Schwartz, K., and Mertz, W. (1957) A glucose tolerance factor and its differentiation from Factor 3. *Arch. Biochem. Biophys. 72*, 515–518.
[30] Mertz, W. Toepfer, E. W., Roginski, E. E., and Polansky, M. M. (1974) Present knowledge of the role of chromium. *Federation Proc. 33*, 2275–2280.
[31] Toepfer, E. W., Mertz, W., Polansky, M. M., Roginski, E. E., and Wolf, W. R. (1977) Preparation of chromium-containing material of glucose tolerance factor activity from Brewer's yeast extracts and by synthesis. *J. Agric. Food Chem. 25*, 162–166.
[32] Barrett, J., O'Brien, P., and de Jesus, J. (1985) Chromium(III) and the glucose tolerance factor. *Polyhedron 4*, 1–14.
[33] Mertz, W. (1998) Chromium research from a distance: From 1959 to 1980. *J. Am. Coll. Nutr. 17*, 544–547.
[34] Vincent, J. B. (2001) The bioinorganic chemistry of chromium(III). *Polyhedron 20*, 1–26.
[35] Yamamoto, A., Wada, O., and Ono, T. (1981) A low-molecular-weight, chromium-binding substance in mammals. *Toxicol. Appl. Pharmacol. 59*, 515–523.
[36] Wada, O., Wu, G. Y., Yamamoto, A., Manabe, S., and Ono, T. (1983) Purification and chromium-excretory function of low-molecular-weight, chromium-binding substances from dog liver. *Environ. Res. 32*, 228–239.
[37] Yamamoto, A., Wada, O., and Ono, T. (1984) Distribution and chromium-binding capacity of a low molecular weight, chromium-binding substance in mice. *J. Inorg. Biochem. 22*, 91–102.
[38] Yamamoto, A., Wada, O., and Ono, T. (1987) Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. *Eur. J. Biochem. 165*, 627–631.
[39] Yamamoto, A., Wada, O., and Suzuki, H. (1988) Purification and properties of biologically active chromium complex from bovine colostrum. *J. Nutr. 118*, 39–45.
[40] Davis, C. M., and Vincent J. B. (1997) Isolation and characterization of a biologically active chromium oligopeptide from bovine liver. *Arch. Biochem. Biophys. 339*, 335–343.
[41] Levina, A., Codd, R., Dillon, C. T., and Lay, P. A. (2003) Chromium in biology: toxicology and nutritional aspects. *Prog. Inorg. Chem. 51*, 145–250.
[42] Gaggelli, E., Berti, F., D'Amelio, N., Valensin, G., Bovalini, L., Paffetti, A., and Trabalzini, L. (2002) Metabolic pathways of carcinogenic chromium. *Env. Health Perspect. 110* (Suppl 5), 733–738.
[43] Feng, W. Y., Qian, Q. F., Ding, W. J., and Chai, Z. F. (2000) Study of chromium speciation in normal and diabetic rats by activable enriched stable isotope technique. *J. Radioanal. Nucl. Chem. 244*, 321–325.
[44] Feng, W., Li, B., Liu, J., Chai, Z., Zhang, P., Gao, Y., and Zhao, J. (2003) Study of chromium-containing proteins in subcellular fractions of rat liver by enriched stable isotopic tracer technique and gel filtration chromatography. *Anal. Bioanal. Chem. 375*, 363–368.
[45] Dinakarpandian, D., Morrissette, V., Chaudhary, S., Amini, K., Bennett, B., and Van Horn, J.D. (2004) An informatics search for the low-molecular weight chromium-binding peptide. *BMC Chem. Biol. 4*, 2.
[46] Yamamoto, A., Wada, O., and Manabe, S. (1989) Evidence that chromium is an essential factor for biological activity of low-molecular-weight, chromium-binding substance. *Biochem. Biophys. Res. Commun. 163*, 189–193.
[47] Davis, C. M., Sumrall, K. H., and Vincent, J. B. (1996) A biologically active form of chromium may activate a membrane phosphotyrosine phosphatase (PTP). *Biochemistry 35*, 12963–12969.
[48] Davis, C. M., and Vincent, J. B. (1997) Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry 36*, 4382–4385.

[49] Vincent, J. B. (2000) Elucidating a biological role for chromium at a molecular level. *Acc. Chem. Res. 33*, 503–510.
[50] Vincent, J. B. (2004) Recent advances in the nutritional biochemistry of trivalent chromium. *Proc. Nutr. Soc. 63*, 41–47.
[51] Cefalu, W. T., and Hu, F. B. (2004) Role of chromium in human health and in diabetes. *Diabetes Care 27*, 2741–2751.
[52] Jacquamet, L., Sun, Y., Hatfield, J., Gu, W., Cramer, S. P., Crowder, M. W., Lorigan, G. A., Vincent, J. B., and Latour, J. M. (2003) Characterization of chromodulin by X-ray absorption and electron paramagnetic resonance spectroscopies and magnetic susceptibility measurements. *J. Am. Chem. Soc. 125*, 774–780.
[53] Glass, M. M., Belmore, K., and Vincent, J. B. (1993) Nuclear magnetic resonance studies of multinuclear chromium assemblies. *Polyhedron 12*, 133–140.
[54] Davis, C. M., Royer, A. C., and Vincent, J. B. (1997) Synthetic multinuclear chromium assembly activates insulin receptor kinase activity: functional model for low-molecular-weight chromium-binding substance. *Inorg. Chem. 36*, 5316–5320.
[55] Mulyani, I., Levina, A., and Lay, P. A. (2004) Biomimetic oxidation of chromium(III): Does the antidiabetic activity of chromium(III) involve carcinogenic chromium(VI)? *Angew. Chem. Int. Ed. 43*, 4504–4507.
[56] Holland, W., Morrison, T., Chang, Y., Wiernsperger, N., and Stith, B. J. (2004) Metformin (glucophage) inhibits tyrosine phosphatase activity to stimulate the insulin receptor tyrosine kinase. *Biochem. Pharmacol. 67*, 2081–2091.
[57] Matthaei, S., Hamann, A., Klein, H. H., Benecke, H., Kreymann, G., Flier, J. S., and Greten, H. (1992) Evidence that metformin increases insulin-stimulated glucose transport by potentiating insulin-induced translocation of glucose transporters from an intracellular pool to the cell surface in rat adipocytes. *Horm. Metabl. Res. Suppl. 26*, 34–41.
[58] Kobayashi, M., Iwanishi, M., Egawa, K., and Shigeta, Y. (1992) Pioglitazone increases insulin sensitivity by activating insulin receptor kinase. *Diabetes 41*, 476–483.
[59] Haase, H., and Maret, W. (2003) Intracellular zinc fluctuations modulate protein tyrosine phosphatase activity in insulin/insulin-like growth factor-1 signaling. *Exp. Cell Res. 291*, 289–298.
[60] May, J. M., and Contoreggi, C. S. (1982) The mechanism of the insulin-like effects of ionic zinc. *J. Biol. Chem. 257*, 4362–4368.
[61] Ueda, M., Robinson, F. W., Smith, M. M., and Kono, T. (1984) Effects of divalent cations on the regulation of insulin-sensitive glucose transport and cAMP phosphodiesterase in adipocytes. *J. Biol. Chem. 259*, 9520–9525.
[62] Mertz, W., Roginski, E. E., and Schwartz, K. (1961) Effect of trivalent chromium complexes on glucose uptake by epididymal fat tissue of rats. *J. Biol. Chem. 236*, 318–322.
[63] Yamamoto, A., Wada, O., Ono, T., Ono, H., Manabe, S., and Ishikawa, S. (1984) Cadmium-induced stimulation of lipogenesis from glucose in rat adipocytes. *Biochem. J. 219*, 979–984.
[64] Baly, D. L., Schneiderman, J. S., and Garcia-Welsh, A. L. (1990) Effect of manganese deficiency on insulin binding, glucose transport, and metabolism in rat adipocytes. *J. Nutr. 120*, 1075–1079.
[65] Goto, Y., and Kidad, K. (1995) Insulin-like action of chromate on glucose transport in isolated rat adipocytes. *Jpn. J. Pharmacol. 67*, 365–368.
[66] Qian, Y., Jing, B. H., Flynn, D. C., Leonard, S. S., Wang, S., Zhang, Z., Ye, J., Chen, F., Wang, L., and Shi, X. (2001) Cr(VI) increases tyrosine phosphorylation through reactive oxygen species-mediated reactions. *Mol. Cell Biochem. 222*, 199–204.
[67] Yurkow, E. J., and Kim, G. (1995) Effects of chromium on basal and insulin-induced tyrosine phosphorylation in H4 hepatoma cells: comparison with phorbol-12-myristate-13-acetate and sodiuim orthovanadate. *Mol. Pharmacol. 47*, 686–695.

[68] Fernandez-Real, J. M., Lopez-Bermejo, A., and Ricart, W. (2002) Cross-talk between iron metabolism and diabetes. *Diabetes 51*, 2348–2354.
[69] Wilson, J. G., Lindquist, J. H., Grambow, S. C., Crook, E. D., and Maher, J. F. (2003) Potential role of increased iron stores in diabetes. *Am. J. Med. Sci. 325*, 332–339.
[70] Scholl, T. O. (2005) Iron status during pregnancy: setting the stage for mother and infant. *Am. J. Clin. Nutr. 81*, 1218S–1222S.
[71] Murakami, K., Okubo, H., and Sasaki, S. (2005) Effect of dietary factors on incidence of type 2 diabetes: a systematic review of cohort studies. *J. Nutr. Sci. Vitaminol. (Tokyo) 51*, 292–310.
[72] Moshtaghie, A. A., Ani, M., and Bazrafshan, M. R. (1992) Comparative binding study of aluminum and chromium to human transferrin. Effect of iron. *Biol. Trace Elem. Res. 32*, 39–46.
[73] Ani, M., and Moshtaghie, A. A. (1992) The effect of chromium on parameters related to iron metabolism. *Biol. Trace Elem. Res. 32*, 57–64.
[74] Clodfelder, B. J., and Vincent, J. B. (2005) The time-dependent transport of chromium in adult rats from the bloodstream to the urine. *J. Biol. Inorg. Chem. 10*, 383–393.
[75] Sargent, T., 3rd, Lim, T. H., and Jenson, R. L. (1979) Reduced chromium retention in patients with hemochromatosis, a possible basis of hemochromatotic diabetes. *Metabolism 28*, 70–79.
[76] Zima, T., Mestek, O., Tesar, V., Tesarova, P., Nemecek, K., Zak, A., and Zeman, M. (1998) Chromium levels in patients with internal diseases. *Biochem. Mol. Biol. Int. 46*, 365–374.
[77] Morris, B. W., MacNeil, S., Hardisty, C. A., Heller, S., Burgin, C., and Gray, T. A. (1999) Chromium homeostasis in patients with type II (NIDDM) diabetes. *J. Trace Elem. Med. Biol. 13*, 57–61.
[78] Ekmekcioglu, C., Prohaska, C., Pomazal, K., Steffan, I., Schernthaner, G., and Marktl, W. (2001) Concentrations of seven trace elements in different hematological matrices in patients with type 2 diabetes as compared to healthy controls. *Biol. Trace Elem. Res. 79*, 205–219.
[79] Rukgauer, M., and Zeyfang, A. (2002) Chromium determinations in blood cells: clinical relevance demonstrated in patients with diabetes mellitus type 2. *Biol. Trace Elem. Res. 86*, 193–202.
[80] Calabrese, E. J., and Baldwin, L. A. (2002) Defining hormesis. *Human Exp. Toxicol. 21*, 91–97.
[81] Southam, C. M. and Erlich, J. (1943) Effects of extracts of western red-cedar heartwood on certain decaying fungi in culture. *Phytopathology 33*, 517–541.
[82] Calabrese, E. J. (2005) Toxicological awakenings: the rebirth of hormesis as a central pillar of toxicology. *Toxicol. Appl. Pharmacol. 204*, 1–8.
[83] Calabrese, E. J. (2005) Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. *Environ. Pollut. 138*, 379–411.
[84] Calabrese, E. J., and Baldwin, L. A. (2003) Ethanol and hormesis. *Crit. Rev. Toxicol. 33*, 407–424.
[85] Calabrese, E. J., and Baldwin L. A. (2003) Peptides and hormesis. *Crit. Rev. Toxicol. 33*, 355–405.
[86] Calabrese, E. J., and Baldwin L. A. (2000) Radiation hormesis: its historical foundations as a biological hypothesis. *Hum. Exp. Toxicol. 19*, 41–75.
[87] Calabrese, E. J., and Baldwin, L. A. (2003) Inorganics and hormesis, *Crit. Rev. Toxicol. 33*, 215–304.
[88] Lamm, S. H., Engel, A., Kruse, M. B., Feinleib, M., Byrd, D. M., Lai, S., and Wilson, R. (2004) Arsenic in drinking water and bladder cancer mortality in the United States: an analysis based on 133 U.S. counties and 30 years of observation. *J. Occup. Environ. Med. 46*, 298–306.

[89] Snow, E. T., Sykora, P., Durham, T. R., and Klein, C. B. (2005) Arsenic, mode of action at biologically plausible low doses: What are the implications for low dose cancer risk? *Toxicol. Appl. Pharmacol. 207(2S)*, 557–564.
[90] Nielson, F. H. (1991) Nutritional requirements for boron, silicon, vanadium, nickel, and arsenic: current knowledge and speculation. *FASEB J. 5*, 2661–2667.
[91] Uthus, E. O. (1992) Evidence for arsenic essentiality. *Environ. Geochem. Health 14*, 55–58.
[92] Mayer, D. R., Kosmus, W., Pogglitsch, H., Mayer, D., and Beyer, W. (1993) Essential trace elements in humans. Serum arsenic concentrations in hemodialysis patients in comparison to healthy controls. *Biol. Trace Elem. Res. 37*, 27–38.
[93] Damelin, L. H., and Alexander, J. J. (2001) Metal-induced hormesis requires cPKC-dependent glucose transport and lowered respiration. *Human Exp. Toxicol. 20*, 347–358.
[94] Mertz, W., Roginski, E. E., and Schwarz, K. (1961) Effect of trivalent chromium complexes on glucose uptake by epididymal fat tissue of rats. *J. Biol. Chem. 236*, 318–322.
[95] Tkeshelashvili, L. K., Shearman, C. W., Zakour, R. A., Koplitz, R. M., and Loeb, L. A. (1980) Effects of arsenic, selenium, and chromium on the fidelity of DNA synthesis. *Cancer Res. 40*, 2455–2460.
[96] Liu, D. H., and Jiang, W. S. (1993) Effects of $Cr^{3+}$ on root growth and cell division of *Allium cepa. Chinese J. Bot. 5*, 34–40.
[97] Pandey, V., Dixit, V., and Shyam R. (2005) Antioxidative responses in relation to growth of mustard (*Brassica juncea* cv. Pusa Jaikisan) plants exposed to hexavalent chromium. *Chemosphere 61*, 40–47.
[98] Liu, P. S., and Lin, M. K. (1997) Biphasic effects of chromium compounds on catecholamine secretion from bovine adrenal medullary cells. *Toxicology 117*, 45–53.

*Chapter 4*

# Effects of chromium(III) as a nutritional supplement*,†

**Henry C. Lukaski**

US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034

## INTRODUCTION

The burgeoning rates of overweight and obesity in the US [1] have prompted individuals to seek weight-control products that are safe, convenient, and effective in promoting weight and fat loss while preserving muscle. These products are also desired because of their purported effects to enhance accretion of muscle or lean mass and to boost performance in strength-dependent activities in response to physical activity and training. One highly advertised and purported weight loss aid is chromium(III) [Cr(III)], particularly in the form of chromium(III) picolinate [$Cr(pic)_3$].

Chromium(III) is a putatively essential mineral that can play a role in carbohydrate and lipid metabolism [2]; it potentiates insulin binding to insulin-sensitive cells and facilitates gene expression [3]. Although inorganic and organic compounds of Cr(III) are available, the predominant chemical form promoted for weight reduction is $Cr(pic)_3$ because of its increased bioavailability compared to other Cr(III) compounds [4, 5].

Sales of Cr(III)-containing supplements are appreciable. From 1996 through 2003, sales of Cr(III) supplements have increased from $65 to $106 million in the United States and represent more than 6% of all mineral supplement sales [6]. Chromium picolinate is the principal supplement of Cr(III) sold, accounting for 80% of all Cr supplement sales. Chromium is sold either individually as $Cr(pic)_3$ or as a component of herbal blends or mixed formulae with vitamins, minerals, or other bioactive ingredients. In addition to tablets and capsules, Cr(III), as $Cr(pic)_3$, is provided in nutrition bars, chewing gum, and sports drinks.

---

* Mention of a trademark or proprietary product does not constitute a guarantee of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

† US Department of Agriculture, Agricultural Research, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

This chapter critically examines the effects of supplemental Cr(III), principally $Cr(pic)_3$, but also other chemical forms of Cr(III) on body weight, body fat, and lean mass of healthy humans under various conditions, including weight maintenance after weight loss, resistance training, and physical conditioning. It evaluates differences in experimental designs including compliance, biochemical responses to Cr(III) supplementation, and limitations in methods of assessment of body composition change. Because variable doses of Cr(III) have been used in the supplementation trials, this review discusses the ranges of amounts of supplemental Cr(III) consumed in comparison to recommended intakes. It concludes with a consensus opinion on the value of Cr(III) supplementation in weight reduction and body composition change.

## EFFECTS OF CHROMIUM SUPPLEMENTATION ON HUMAN BODY COMPOSITION AND PHYSICAL FUNCTIONS

### Untrained Adults

The impetus for research to determine the effects of supplemental Cr(III) came from two studies of healthy men [7]. Ten men aged 18–21 years enrolled in a weight training class were randomized to receive either 200 μg Cr(III) as $Cr(pic)_3$ or placebo (calcium phosphate) for 40 days. Men were instructed to follow usual dietary and physical activity patterns while participating in two weight training sessions weekly. Body weight and composition, assessed by using skin-fold thicknesses and body circumferences, were measured before and after training. After completion of the resistance training, the body weight of the men supplemented with $Cr(pic)_3$ increased 2.2 kg with a slight increase in body fatness (% fat). Lean body mass was reported to increase significantly by 1.6 kg. Placebo-treated men also increased body weight (1.25 kg), but body fatness increased significantly (1.1%) with a negligible increase in lean body mass (0.4 kg).

Subsequent studies examined the interaction of $Cr(pic)_3$ supplementation and strength training failed to demonstrate a benefit of Cr(III) on changes in body structure and function. Hasten et al. [8] enrolled 59 college-age students (22 women and 37 men) in a 12-week weightlifting program (3 sessions/week for 40 min/session). Volunteers were randomized by sex and received either 200 μg Cr(III) as $Cr(pic)_3$ or placebo (lactose); compliance was monitored every 3 weeks. Body weight, skinfold thicknesses (women: triceps, suprailiac, and thigh; men: pectoral, abdominal, and thigh), and body circumferences (chest, arm, and thigh) were measured at randomization and at weeks 3, 6, 9, and 12 of training. After 12 weeks, the women supplemented with $Cr(pic)_3$ gained significantly more weight (2.5 kg) than the other groups (0.6–1.3 kg). No treatment effects on body composition measures were found. The mean increase of the sum of three circumferences was 2.3 cm, and the mean decrease in the sum of three skinfold thicknesses was 4.8 cm. No effects of supplemental Cr(III) on strength measures were identified, although the men gained more strength than the women. Thus, the only discernable effect of $Cr(pic)_3$ supplementation was a gain in body weight (4.3% vs. 1%) of the female participants.

Hallmark et al. [9] also determined the effects of $Cr(pic)_3$ supplementation on muscular strength and body composition of young men. Sixteen untrained collegiate men were matched by initial strength assessments and then randomized to receive treatments

of either 200 μg Cr(III) as $Cr(pic)_3$ or placebo. Analysis of the capsules showed the Cr supplement and placebo contained 188 and 0.9 μg Cr(III), respectively. The men participated in progressive resistance training 3 times per week for 12 weeks. Usual dietary Cr intake was estimated to be 36 μg/day. Urinary Cr excretion was significantly increased in men supplemented with $Cr(pic)_3$ compared to placebo. Upper- and lower-body strength increased significantly after training in both groups (23–35%) with no effect of Cr(III) supplementation. Similarly, no significant changes in body composition, determined by hydrodensitometry, were found after weight training.

Lukaski et al. [10] expanded the scope of research on the effects of supplemental Cr(III) on body composition and strength gain by examining different chemical forms of Cr(III), whole-body and regional muscle mass, and Cr in blood and urine. Thirty-six men (19–29 years) participated in a double-blind supplementation trial. They were matched by admission body composition, body build (somatotype), strength measures, and fasting serum Cr concentrations into three treatment groups [placebo (lactose), Cr(III) chloride ($CrCl_3$), and $Cr(pic)_3$]. Chemical analyses of the treatment capsules revealed that the placebo, $CrCl_3$, and $Cr(pic)_3$ contained 0.1, 180, and 172 μg Cr, respectively. The men trained 5 sessions/week, 60 min/session, for 8 weeks. Training intensities were increased every 2 weeks based on individual assessments of strength. Compliance was 99% for all training sessions and capsule ingestion. Chromium supplementation did not significantly affect body weight or composition, determined by using dual X-ray absorptiometry. Body weight, circumferences, mesomorphy, and muscle mass increased significantly, and body fatness decreased with resistance training in all groups. Strength in all exercises increased significantly (15–40%) depending on muscle mass involved with no Cr(III) treatment effect. Average Cr intake from food was estimated to be 40 μg/day in the 36 men. Serum Cr concentrations increased significantly in the men supplemented with Cr(III) with no difference ascribed to the source of Cr. Similarly, urinary Cr excretion increased significantly with Cr(III) supplementation independently of Cr(III) compound. These findings indicate that supplemental Cr(III), regardless of the chemical form, had no effects on muscular strength gain or body composition change in men participating in controlled resistance training.

An increased dose of supplemental Cr(III) failed to show any benefit. Twenty male and female college students participating in resistance and aerobic training for 13 weeks were randomized to groups supplemented with either 1000 μg Cr(III) as $Cr(pic)_3$ or placebo [11]. Exercise alone or coupled with $Cr(pic)_3$ did not produce significant changes in strength or lean body mass.

**Body composition and performance in athletes**

Chromium(III) has been touted as a possible ergogenic aid for athletes. The generalized use of Cr(III) supplements, particularly $Cr(pic)_3$, was fostered by reports that physically active people have Cr(III) intakes equivalent to less than half of the amount recommended and that physical stressors, such as exercise, increase losses of Cr(III), thus, exacerbating any suboptimal Cr(III) status [12, 13].

Evans [7] reported the first study of $Cr(pic)_3$ supplementation [200 μg Cr(III)] and strength training in athletes. Forty-two collegiate football players participated, but only 31 completed the study. Baseline body weight and composition data were not reported. Body fatness (2.7% vs 0.5%) decreased significantly and lean body mass

(1.8% vs 0.9 kg) increased significantly, as determined by using anthropometry, among the men supplemented with $Cr(pic)_3$ compared to placebo after 14 days of supplementation and strength training. After 42 days of supplementation and resistance training, the athletes consuming $Cr(pic)_3$ had lost 1.2 kg body weight with a concomitant loss of 3.4 kg of fat mass and a gain of 2.6 kg of lean body mass. In contrast, men supplemented with placebo lost 1 kg of body fat mass and gained 1.8 kg of lean tissue. Although the reported differences by treatment were described as significantly different, no variability estimates were included.

Forty male football players participated in another double-blind supplementation study in which Cr(III) (200 μg/day as $Cr(pic)_3$) and placebo were the treatments [14]. All subjects participated in a 9-week supervised off-season training program, including intensive weightlifting (4 days/week) and running (2 days/week) sessions. Thirty-six men completed all aspects of the study. Urinary Cr(III) excretion increased five-fold among the men supplemented with $Cr(pic)_3$. Body composition improved with training in both treatment groups with no significant effect of Cr(III) supplementation on gain of muscle mass. Surprisingly, strength did not change significantly after training in either group. This unexpected finding was not discussed by the authors.

Walker et al. [15] evaluated the effects of $Cr(pic)_3$ supplementation [200 μg Cr(III)] daily for 14 weeks of pre-season resistance training and conditioning program during which the wrestlers were not attempting to lose weight. Twenty NCAA division I wrestlers were assigned to treatments by using a stratified random sampling technique based on weight classification. The treatments included Cr (III), placebo, and control.

Body weight, body fatness, and fat mass decreased in all groups with no effect of Cr(III) supplementation. Lean body mass was maintained despite a 10% decrease in self-reported energy intake. Interestingly, dietary analysis indicated that Cr(III) intake decreased significantly in each treatment group with average intakes of 30–40 μg/day during training. All groups increased strength, neuromuscular performance, and metabolic performance with no effect of Cr(III) supplementation.

Only one study has evaluated the effects of Cr(III) supplementation on body composition adaptation to resistance training in women. Fifteen female softball players from a division I program enrolled in a double-blind supplementation trial [16]. Eight players received 500 μg Cr(III) as $Cr(pic)_3$, and 7 women received placebo for 6 weeks while participating in a strength training program (3 sessions/week). Urinary Cr(III) excretion increased significantly in the $Cr(pic)_3$ supplemented women. Cr(III) supplementation did not affect body weight or composition estimated by using hydrodensitometry. Muscular strength increased significantly with no differences between the treatment groups.

In contrast to the previous findings, one study reported beneficial effects of $Cr(pic)_3$ supplementation. Forty collegiate swimmers participated in a double-blind study of effects of daily Cr(III) supplementation [400 μg Cr(III) as $Cr(pic)_3$] for 24 weeks during a competitive season [17]. Swimmers supplemented with $Cr(pic)_3$ significantly increased lean body mass (3.3%), decreased body fatness (6.4%), and fat weight (4.6%) compared to the placebo-treated swimmers. Female swimmers responded more to the Cr(III) supplementation than did the male swimmers. Importantly, the most pronounced impact of Cr(III) occurred during the last 12 weeks of the competitive season. The authors cautioned against over-emphasis of the positive effects of Cr(III) supplementation. They remarked that the beneficial changes in body composition may be a result of increased

training intensity, and not supplemental Cr(III). The authors suggested that $Cr(pic)_3$ supplementation may require very high intensity aerobic exercise to elicit body fat loss and muscle gain.

**Weight loss and maintenance**

Obesity generally is associated with increased circulating insulin concentrations that are directly related to body fat content. The purported link between Cr(III) and facilitation of insulin action stimulated investigators to determine the effects of supplemental Cr(III) on weight loss and maintenance with an emphasis on reduction of body fat and preservation of lean body mass in a variety of situations. Interestingly, the amount of supplemental Cr(III) used far exceeded the 200 μg/day used in previous studies. Apparently, the investigators speculated that weight loss increased Cr(III) needs to ameliorate any deficits in insulin utilization.

Initial examination of the benefits of supplemental Cr(III) in weight loss came from Evans and Pouchnik [18]. Twelve men and twelve women participating in an aerobics class were randomized to receive $Cr(pic)_3$ or Cr(III) nicotinate [$Cr(nic)_3$] placebo. The men received 400 μg while the women were supplemented with 200 μg Cr(III) daily for 8 weeks. Body composition was estimated by using bioelectrical impedance analysis. Men supplemented with $Cr(pic)_3$ increased lean body weight by 2.1 kg, which was similar to the change found in the men receiving $Cr(nic)_3$. Females supplemented with $Cr(pic)_3$ also gained lean body weight (1.8 kg), a change equivalent to the gain observed in the females supplemented with $Cr(nic)_3$. These findings suggest similar benefits of Cr(III) as the picolinate compared to the nicotinate compound.

Active duty US Navy personnel are required to meet standards for body fatness. Failure to meet these standards necessitates physical conditioning. Trent and Thieding-Cancel [19] enrolled 95 Navy personnel with excessive body fatness. Seventy-nine men and sixteen women (mean age 30.3 years) participated in a double-blind, placebo-controlled study; they were randomized to receive either 400 μg Cr (III) daily as $Cr(pic)_3$ or placebo and assigned to aerobic exercise training at least 30 min/day, 3 days/week for 16 weeks. Regardless of sex, there was a significant decrease in body weight (1.2 kg) and body fatness (0.6%) determined by anthropometry with no significant change in lean body mass (−0.3 kg). No independent effect of Cr(III) supplementation on weight or fat loss was shown.

The effect of Cr(III) in different organic compounds has been evaluated in conjunction with exercise training in obese adults. Grant et al. [20] studied 43 obese women with body fatness of 25–45% and aged 18–35 years. The women participated in a cross-training exercise program including step aerobics, cycling, and resistance training for 9 weeks. They were randomly assigned to one of four treatment groups: $Cr(pic)_3$ without exercise, exercise training with $Cr(pic)_3$, exercise training with placebo (inert ingredients), and exercise training with $Cr(nic)_3$. Cr(III) was consumed as two capsules daily; each was reported to contain 200 μg. Body weight increased significantly only in the non-exercising women supplemented with $Cr(pic)_3$. Thus, Cr(III) supplementation was not effective in reducing body weight. There were neither treatment effects on body composition, assessed by using hydrodensitometry, nor on fasting glucose or insulin concentrations. However, in response to an oral glucose tolerance test, the area under the insulin curve was significantly reduced only in the women treated with $Cr(nic)_3$ and

exercise. These findings suggest that $Cr(nic)_3$, not $Cr(pic)_3$, may be beneficial in risk factor modification in obese adults.

Volpe et al. [21] also evaluated the effects of $Cr(pic)_3$ added to a controlled exercise program on body composition and some biochemical parameters in obese women during weight loss. Forty-four premenopausal women, aged 27–51 years with a body mass index (BMI) ranging from 27 to 41 $kg/m^2$, were matched by BMI then randomized to one of two treatment groups: $Cr(pic)_3$ [400 μg Cr(III)] or placebo ($Ca_2PO_4$) for 12 weeks. All women participated in the exercise component of the weight loss program, including 30 min of resistance training and 30 min of moderate intensity walking daily, 2 days/week. Thirty-seven of the volunteers (84%) completed the study; 20 women in the $Cr(pic)_3$ and 17 women in the placebo group. Neither body composition, determined by using hydrodensitometry, nor sum of the circumferences of the waist and hips were affected by $Cr(pic)_3$ supplementation. Plasma Cr concentration and urinary Cr excretion increased significantly with $Cr(pic)_3$ supplementation. Muscular strength significantly increased regardless of treatment.

Two additional studies failed to provide reasonable evidence of a beneficial effect of Cr(III) in weight loss. One-hundred-and-fifty-four obese adults participated in a randomized, double-blind, placebo-controlled trial to determine the effects of $Cr(pic)_3$ supplements on the composition of weight lost [22]. Volunteers were assigned to treatment groups that received Cr(III), 200 or 400 μg/day, or placebo for 72 days. Supplemental Cr(III) was contained in a proprietary drink composed of protein and carbohydrate. Volunteers were asked to consume at least two serving daily, but there was no control of the volume of the drink consumed by a subject. No instruction regarding dietary practices or physical activity was provided to the participants. Body composition was determined by using hydrodensitometry before and after treatment. Compared to the placebo group, significant decreases in body weight, fat weight, and body fatness were found for the groups supplemented with 200 and 400 μg Cr(III); there was no effect of amount of Cr(III) on these variables. Treatment did not significantly affect lean body mass. The authors calculated an unvalidated variable, body composition improvement index, that accounts for fat loss (positive effect) and reduction of lean body mass (negative effect) that occurred during the trial. They concluded that Cr(III) supplementation facilitated significantly more positive changes in body composition compared to the results from the placebo. No significant differences were found in the body composition improvement index of the groups supplemented with 200 or 400 μg of Cr(III) daily.

In a second study, 122 obese adults (17 men and 105 women; mean age 42.3 years) were randomized to receive a capsule containing either 400 μg Cr(III) as $Cr(pic)_3$ or placebo for 90 days [23]. Subjects monitored themselves and reported daily caloric intake and energy expenditure to fitness instructors. Changes in body composition, determined by dual X-ray absorptiometry, included significant reductions in body weight and body fat with effect of Cr(III) supplementation. Body fat, however, decreased significantly more in the subjects supplemented with $Cr(pic)_3$. Lean body weight decreased but not significantly in both treatment groups. The authors further adjusted the body composition data. They calculated additional change in fat weight on the basis that 3500 kcal energy expenditure reflected a 1 lb loss of body fat. After controlling self-reported differences in energy intake and output, the subjects with the Cr(III)-supplemented diets, as compared

to the placebo group, lost significantly more weight (7.8 vs. 1.8 kg) and fat weight (7.7 vs. 1.5 kg) without loss of lean body mass.

Interpretation of the data from the studies of Kaats et al. [22, 23] is complicated by some key issues. Use of the calculation of the body composition improvement index rather than actual assessments of body composition is suspicious. Also, lack of control of or assessment of Cr(III) intake and of recording accurate energy intake and expenditure limit the interpretation of the findings. Similarly, calculation of loss of fat based on 3500-kcal energy expenditure associated with physical activity fails to acknowledge homeostatic adaptation in energy metabolism and produces dubious conclusions. Therefore, conclusions from these results should be viewed with caution.

A double-blind, placebo-controlled pilot study evaluated the effects of Cr(III)-bound niacin on weight loss and its composition in obese women [24]. Twenty African-American women were randomized to two groups that received either a total of 600 μg [Cr(III)] daily or placebo in a cross-over designed trial. One group began with 200 μg/day Cr(III) supplementation administered 3 times daily for 2 months, then a 1 month wash-out, followed by 2 months of placebo. Concurrently, the other group had a reversed treatment design. Body composition was evaluated by using bioelectrical impedance analysis. In the group treated with placebo first, body fat weight decreased significantly more with the Cr(III)-bound niacin compared to placebo. Also, significantly less lean body weight was lost with Cr(III) compared to placebo. In contrast, the group supplemented first with Cr(III)-bound niacin lost significantly more fat and less lean body weight during the placebo compared to Cr(III) supplementation. These findings suggest a beneficial effect of Cr(III)-bound niacin on fat loss and preservation of lean body weight. Also, this effect of Cr(III)-bound niacin on body composition apparently persists after cessation of its consumption.

Attempts to demonstrate the benefit of $Cr(pic)_3$ on the maintenance of weight have not been successful. Twenty-one obese patients who successfully completed an 8-week very low calorie diet were supplemented with 200 μg Cr(III), either as $Cr(pic)_3$ or Cr-enhanced yeast daily, or placebo for an 18-week weight-maintenance period [25]. Body composition was assessed by using skin-fold thicknesses. Although body weight and body fatness were not influenced by treatment, lean body mass significantly increased in the group supplemented with $Cr(pic)_3$ compared to the other treatments. This finding suggests that Cr(III) promotes muscle retention, if not gain, during weight maintenance after weight loss.

Thirty-three obese women who completed a 2-week verylow calorie diet were randomized to receive a supplement containing $Cr(pic)_3$ [200 μg Cr(III)], fiber (20 g), carbohydrate (50 g), and caffeine (100 mg), or placebo for 16 months [26]. The amount and course of the weight relapse was similar between the groups with no differences in body composition by treatment. In contrast to the previous report [25], these findings indicate that Cr(III), as a component of a supplement, was not useful in maintaining weight loss in weight-reduced adults.

## Compositional and Functional Effects of Cr(III) Supplementation in Older Adults

Advancing age has been associated with significant decreases in muscle mass and impairments in ambulatory function as well as decreased insulin sensitivity. Because of

its putative role in anabolism as a potentiator of insulin action [27], supplementation of Cr(III) independently and in conjunction with weight training has been hypothesized to increase muscle mass in the elderly.

Nineteen healthy men and women, 63–77 years of age, were randomly assigned in a double-blind study and received either 1000 μg Cr(III) as $Cr(pic)_3$ or placebo for 8 weeks [28]. Serum Cr(III) concentrations increased significantly with $Cr(pic)_3$ supplementation. Body composition and insulin sensitivity were unchanged in both groups. Thus, $Cr(pic)_3$ alone did not change body composition or improve insulin sensitivity in healthy elder men and women.

The interaction of supplemental Cr(III) and resistance training on body composition and strength gain has been assessed in older men and women. Eighteen healthy men, 56–69 years of age, were randomly assigned (double-blind) to groups that consumed either 924 μg Cr(III) as $Cr(pic)_3$ or placebo for 13 weeks and participated in a supervised resistance training program of 2 sessions weekly [29]. For 5-day sequences during each 3-week testing periods, the men consumed controlled diets designed to maintain constant Cr(III) intake (~60 and ~100 μg Cr(III) daily in a 2-day rotating menu). Lean body mass, muscle mass (estimated by urinary creatinine excretion), and rates of strength gain increased independently of $Cr(pic)_3$ supplementation.

A similar study was conducted in women, aged 54–74 years. [30] Seventeen sedentary older women were randomized to groups that received either 924 μg Cr(III) as $Cr(pic)_3$ or placebo for 13 weeks and participated in a supervised resistance training program (2 training sessions/week). Resistance training significantly increased muscle strength (8–34%) of the muscle groups trained; these responses were not affected by $Cr(pic)_3$ supplementation. Fat-free mass and body fatness were not changed by resistance training in the weight-stable women regardless of $Cr(pic)_3$ supplementation.

## Cr(III): ANABOLIC OR CATABOLIC?

An enigma persists regarding the biological role of Cr on body weight and composition. If Cr(III) potentiates insulin action [3, 27], it should act as an anabolic agent. Thus, Cr(III) should up-regulate protein synthesis and promote gain of muscle and lean body mass. However, the mechanism by which supplemental Cr(III) promotes weight and fat loss, as hypothesized [7], is unknown. One might suggest that Cr(III) has an unexplained effect on increasing energy expenditure. Conversely, volunteers supplemented with Cr(III), as $Cr(pic)_3$, have reduced energy intake while increasing protein intake. Thus, acceptance of the findings of decreased body weight and/or fat with $Cr(pic)_3$ supplementation should be viewed as dubious until confirmed with data from a study of controlled energy intake.

## Chromium(III) SUPPLEMENTATION: NUTRITION OR PHARMACOLOGY

One concern about the use of Cr(III) supplements is the amount of the dose consumed. Before 2001, the recommendation for dietary Cr(III) intake was 50–200 μg/day for adults [31]. The range was based on the absence of signs of Cr deficiency among

people consuming 50 μg/day and a safe upper limit of intake of 200 μg/day. If absorption of Cr(III) is 0.5% [32], then the amount of Cr available from a 50 μg intake is 0.25 μg. There are no estimates of absorption of doses exceeding 50 μg. Thus, supplementation trials initiated before 2001 used the upper limit of the safe and adequate intake of Cr to maximize the opportunity to identify any beneficial effects of supplemental Cr.

After 2001, the recommendation for Cr intake was revised [33]. A lack of experimental evidence to establish the average daily intake to meet the needs of half of the US adult population resulted in the use of estimated intakes of healthy men and women. Thus, the recommended intake is 35 and 25 μg/day for men and women, respectively. No tolerable upper limit of Cr has been established.

Evaluation of the doses of supplemental Cr(III) is hampered by the lack of data from controlled studies of potentially biologically adverse effects of increasing doses of Cr(III). Results of national nutritional surveys indicate that the 99 percentile of total Cr intake, diet plus supplements, is 200 μg/day [33]. Thus, one may conclude that doses exceeding 200 μg/day should be viewed as pharmacological. Importantly, no studies of Cr(III) supplementation with doses exceeding 200 μg/day have shown beneficial effects on body weight and composition. Individual case reports of adverse effects of consumption of Cr(III), particularly $Cr(pic)_3$, at doses exceeding 200 μg/day have been summarized [34, 3].

## CONSENSUS ON Cr(III) SUPPLEMENTS FOR WEIGHT REDUCTION, BODY COMPOSITION CHANGE, AND STRENGTH GAIN

Consensus for effects of Cr(III) supplementation is conjectural because of inconsistent findings attributable to problems in experimental design. Many studies utilize samples of convenience and, thus, suffer from inadequate sample sizes and statistical power to test hypotheses. This limitation is significant because of the large within-subject variability in responses to supplementation and other interventions. There is a general lack of assessment of energy and Cr intakes before and during supplementation, which limits interpretation of data on changes in body weight and composition. Determination of compliance regarding supplement consumption and adherence to training programs is also lacking in many studies. More problematic, however, is the use of body composition assessment methods, such as anthropometry and bioelectrical impedance analysis that are not sufficiently sensitive to reliably detect the anticipated modest changes in body fat or lean mass [35, 36]. These concerns fuel the controversy surrounding the value of Cr(III) supplementation as a weight loss aid.

Despite these limitations, the preponderance of the literature reports little or no effect of Cr(III) supplementation, as $Cr(pic)_3$, compared to placebo in facilitating weight loss or promoting propitious changes in body fat and/or lean body mass (Table 1). Consistent with these findings, a 4-month supplementation trial with 200 and 1000 μg Cr(III) as $Cr(pic)_3$ reported no change in BMI of 180 adults with type 2 diabetes despite significant reductions in glycated hemoglobin [37]. Thus, Cr(III) supplementation should not be viewed as a weight loss aid in adults. Similar conclusions have been reported by other reviewers [34, 38, 39].

Table 1
Effects of chromium (Cr) supplementation of body weight and composition[a]

| Subjects[b] | Form[c] and Dose (μg/d) | Placebo | Time (wk) | Exercise[d] | Compliance | Method[e] | Results | Chromium | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Blood | Urine | |
| Untrained adults | | | | | | | | | | |
| 10 M | $Cr(pic)_3$, 200 | Yes | 5–6 | Resistance | No | SF, Circ | ↑LBM | – | – | 7 |
| 37 M, 22 F | $Cr(pic)_3$, 200 | Yes | 12 | Resistance | Yes | SF, Circ | M: no effects<br>F: ↑body weight | – | – | 8 |
| 16 M | $Cr(pic)_3$, 188[f] | Yes | 12 | Resistance | Yes | UWW | No effects | – | ↑ | 9 |
| 36 M | $Cr(pic)_3$, 172[f] | Yes | 8 | Resistance | Yes | DXA | No effects | ↑ | ↑ | 10 |
| | $CrCl_3$, 180[f] | Yes | 8 | Resistance | Yes | DXA | No effects | ↑ | ↑ | 10 |
| 35 M, F | $Cr(pic)_3$, 1000 | Yes | 13 | Resistance, | Yes | SF, Circ | No effects | | | 11 |
| Athletes | | | | | | | | | | |
| 31 M | $Cr(pic)_3$, 200 | Yes | 6 | Resistance | No | SF, Circ | ↑LBM, ↓Fat | – | – | 7 |
| 36 M | $Cr(pic)_3$, 200 | Yes | 9 | Resistance | No | UWW | No effects | – | ↑ | 14 |
| 20 M | $Cr(pic)_3$, 200, | Yes | 14 | Resistance, Aerobic | Yes | UWW | No effects | – | – | 15 |
| 15 F | $Cr(pic)_3$, 500 | Yes | 6 | Resistance | Yes | UWW | No effects | – | ↑ | 16 |
| 20 M, 20 F | $Cr(pic)_3$, 400 | Yes | 24 | Swimming | No | UWW | ↑LBM, ↓Fat | – | – | 17 |
| Weight loss and maintenance | | | | | | | | | | |
| 12 M | $Cr(pic)_3$, 400<br>$Cr(nic)_3$, 400 | Yes | 12 | Aerobics | No | BIA | No effects | – | – | 18 |
| 12 F | $Cr(pic)_3$, 200<br>$Cr(nic)_3$, 200 | Yes | 12 | Aerobics | No | BIA | No effects | – | – | 18 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 79 M, 16 F | $Cr(pic)_3$, 400 | Yes | 16 | Resistance, Aerobic | No | SF, Circ | No effects | – | – | 19 |
| 43 F | $Cr(pic)_3$, 200 | Yes | 9 | Resistance, Aerobic | Yes | UWW | ↑Weight | – | – | 20 |
| | $Cr(nic)_3$, 200 | Yes | 9 | Resistance, Aerobic | Yes | UWW | No effects | – | – | 20 |
| 44 F | $Cr(pic)_3$, 400 | Yes | 12 | Resistance, Aerobic | Yes | UWW | No effects | ↑ | ↑ | 21 |
| 154 M, F | $Cr(pic)_3$, 200, 400 | Yes | 11 | None | Yes | UWW | ↑LBM, ↓Fat | – | – | 22 |
| 17 M, 105 F | $Cr(pic)_3$, 400 | Yes | 13 | Undefined | Yes | DXA | ↓Fat | – | – | 23 |
| 20 F | Cr-niacin, 600 | Yes | 8 | None | Yes | BIA | ↑LBM | – | – | 24 |
| 21 M, F | Cr-yeast 200 | Yes | 18 | Undefined | No | SF | ↑LBM | – | – | 25 |
| 33 F | $Cr(pic)_3$, 200, | Yes | 64 | None | No | SF, Circ | No effects | – | – | 26 |
| Older adults | | | | | | | | | | |
| 10 M, 9 F | $Cr(pic)_3$, 1000 | Yes | 8 | None | Yes | DXA | No effects | – | – | 28 |
| 18 M | $Cr(pic)_3$, 964[f] | Yes | 13 | Resistance | Yes | UWW | No effects | – | ↑ | 29 |
| 17 F | $Cr(pic)_3$, 964[f] | Yes | 13 | Resistance | Yes | UWW | No effects | – | ↑ | 30 |

[a] LBM = lean body mass.
[b] M = male; F = female.
[c] $Cr(pic)_3$ = chromium picolinate; $Cr(nic)_3$ = chromium nicotinate; $CrCl_3$ = chromium chloride.
[d] Resistance = strength training; Aerobic = running or walking; None = no exercise.
[e] SF, Circ = skinfold thicknesses and circumferences; BIA = bioelectrical impedance analysis; DXA = dual x-ray absorptiometry; UWW = hydrodensitometry.
[f] Analyzed value.

## SOME RESOLUTION TO THE CONTROVERSY

A recent study has overcome many of these limitations and, thus, provides a clearer view of the effects of Cr(III) supplementation on body weight and composition [40]. Eighty-three premenopausal women were matched by BMI and randomized in a double-blind study to receive one of three treatment groups: Cr(III) supplement with 200 μg (analyzed value: 190 μg) Cr(III) as $Cr(pic)_3$, picolinic acid (equivalent to that in the matched $Cr(pic)_3$; 1700 mg), and placebo (lactose). After assessment of individual energy needs, the women consumed only food and beverages provided for 12 weeks. The study tested the hypothesis that Cr(III) supplementation decreases body weight and alters body composition when energy intake is constant. Body weight was maintained within 2% of admission values. Body composition, determined at admission and 4 weeks by using dual X-ray absorptiometry, did not change significantly. Thus, under the conditions of constant energy intake, supplemental Cr(III) neither affected body weight nor composition.

## CONCLUSION

Despite a ruling by the US Federal Trade Commission [41] that there is no basis for claims that Cr(III), as $Cr(pic)_3$, promotes weight loss and fat loss in humans, sales of $Cr(pic)_3$ continue to grow [6]. Results of this review reinforce this ruling and indicate that any benefit of this supplement as a weight loss aid is negligible.

## REFERENCES

[1] Hedley, A. A., Ogden, C. L., Johnson, C. L., Carroll, M. D., Curtin, L. R., and Flegal, K. M. (2004) Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *JAMA 291*, 2847–2850.

[2] Anderson, R. A. (1998) Chromium, glucose intolerance and diabetes. *J. Am. Coll. Nutr. 17*, 548–555.

[3] Vincent, J. B. (2004) Recent developments in the biochemistry of chromium(III). *Biol. Trace Elem. Res. 99*, 1–16.

[4] Olin, K. L., Stearns, D. M., Armstrong, W. H., and Keen, C. L. (1994) Comparative retention/absorption of chromium ($^{51}Cr$) from $^{51}Cr$ chloride, $^{51}Cr$ nicotinate and $^{51}Cr$ picolinate in a rat model. *Trace Elem. Electrolytes 11*, 182–186.

[5] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Gautschi, K. (1996) Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. *J. Trace Elem. Exp. Biol. 9*, 11–25.

[6] Nutrition Business Journal. (2005) Sports Nutrition and Weight Loss. San Diego, CA.

[7] Evans, G. W. (1989) The effect of chromium picolinate on insulin controlled parameters in humans. *Int. J. Biosci. Med. Res. 11*, 163–180.

[8] Hasten, D. L., Rome, E. P., Franks, D. D., and Hegsted, M. (1997) Effects of chromium picolinate on beginning weight training students. *Int. J. Sports Nutr. 2*, 343–350.

[9] Hallmark, M. A., Reynolds, T. H., DeSouza, C. A., Dotson, C. O., Anderson, R. A., and Rogers, M. A. (1996) Effects of chromium and resistive training on muscle strength and body composition. *Med. Sci. Sports Exerc. 28*, 139–144.

[10] Lukaski, H. C., Bolonchuk, W. W., Siders, W. A., and Milne, D. B. (1996) Chromium supplementation and resistance training: Effects on body composition, strength, and trace element status of men. *Am. J. Clin. Nutr. 63*, 954–965.

[11] Boyd, S. G., Boone, B. E., Smith, A. R., Conners, J., and Dohm, G. L. (1998) Combined dietary chromium picolinate supplementation and an exercise program leads to a reduction of serum cholesterol and insulin in college-aged subjects. *J. Nutr. Biochem. 9*, 471–475.

[12] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Deuster, P. A. (1988). Exercise effects on chromium excretion of trained and untrained men consuming a constant diet. *J. Appl. Physiol. 64*, 249–252.

[13] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Thorp, J. W. (1991) Effects of carbohydrate loading and underwater exercise on circulating cortisol, insulin and urinary losses of chromium and zinc. *Eur. J. Appl. Physiol. Occup. Physiol. 63*, 146–150.

[14] Clancy, S. P., Clarkson, P. M., Cheke, M. E. D., Nosaka, K., Freedson, P. S., Cunningham, J. J., and Valentine, B. (1994) Effects of chromium picolinate supplementaion on body composition, strength, and urinary chromium loss in football players. *Int. J. Sport Nutr. 4*, 142–153.

[15] Walker, L. S., Bemben, M. G., Bemben, D. A., and Khenans, A. W. (1998) Chromium picolinate effects on body composition and muscular performance in wrestlers. *Med. Sci. Sports Exerc. 30*, 1730–1737.

[16] Livolsi, J. M., Adams, G. M., and Laguna, P. L. (2001) The effect of chromium picolinate on muscular strength and body composition in women athletes. *J. Strength Cond. Res. 15*, 161–166.

[17] Bulbulian R., Pringle, D. D., and Liddy, M. S. (1996) Chromium picolinate supplementation in male and female swimmers. *Med. Sci. Sports Exerc. 28*, Suppl, S111.

[18] Evans, G. W., and Pouchnik, D. J. (1993) Composition and biological activity of chromium-pyridine complexes. *J. Inorg. Biochem. 49*, 177–187.

[19] Trent, L. K., and Thieding-Canel, D. (1995) Effects of chromium picolinate on body composition. *J. Sports Med. Phys. Fitness 35*, 273–280.

[20] Grant, K. E., Chandler, R. M., Castle, A. L., and Ivy, J. L. (1997) Chromium and exercise training: effect on obese women. *Med. Sci. Sports Exerc. 29*, 992–998.

[21] Volpe, S. L., Huang, H. W., Larpadisorn, K., and Lesser, I. I. (2001) Effect of chromium supplementation and exercise on body composition, resting metabolic rate and selected biochemical parameters in moderately obese women following an exercise program. *J. Am. Coll. Nutr. 20*, 293–306.

[22] Kaats, G. R., Blum, K., Fisher, J. A., and Adelman, J. A. (1996) Effects of chromium supplementation on body composition: a randomized, double-masked, placebo-controlled study. *Curr. Ther. Res. 57*, 747–456.

[23] Kaats, G. R., Blum, K., Pullin, D., Keith, S. C., and Wood, R. (1998) A randomized, double-masked, placebo-controlled study of the effects of chromium picolinate supplementation on body composition: a replication and extension of a previous study. *Curr. Ther. Res. 59*, 379–388.

[24] Crawford, V., Scheckenbach, R., and Preuss, H. G. (1999) Effects of niacin-bound chromium supplementatioin on body compsotion in overweight African-American women. *Diabetes Obesity Metabol. 1*, 331–337.

[25] Bahadori, B., Wallner, S., Schneider, H., Wascher, T. C., and Toplak, H. (1997) Effect of chromium yeast and chromium picolinate on body composition of obese, non-diabetic patients during and after a formula diet. *Acta Med. Austriaca 24*, 185–187.

[26] Pasman, W. J., Westerterp-Plantenga, M. S., and Saris, W. H. M. (1997) The effectiveness of long-term supplementation of carbohydrate, chromium, fibre, and caffeine on weight maintenance. *Int. J. Obes. 21*, 1143–1151.

[27] Lefavi, R. G., Anderson, R. A., Keith, R. E., Wilson, G. D., McMillan, J. L., and Stone, M. H. (1992) Efficacy of chromium supplementation in athletes: emphasis on anabolism. *Int. J. Sport Nutr. 2*, 111–122.

[28] Amato, P., Morales, A. J., and Yen, S. S. C. (2000) Effects of chromium picolinate supplementation on insulin sensitivity, serum lipids, and body composition in healthy, non-obese older men and women. *J. Gerontol. 55A*, M260–M263.

[29] Campbell, W. W., Joseph, L. J. O., Davey, S. L., Cyr-Campbell, D., Anderson, R. A., and Evans, W. J. (1999). Effects of resistance training ands chromium picolinate on body composition and skeletal muscle in older men. *J. Appl. Physiol. 86*, 29–39.

[30] Campbell, W. W., Joseph, L. J. O., Anderson, R. A., Davey, S. L., Hinton, J., and Evans, W. J. (1999). Effects of resistance training and chromium picolinate on body composition and muscle size in older women. *Int. J. Sport Nutr. Exerc. Metabol. 12*, 125–135.

[31] National Research Council (1989) *Recommended Dietary Allowances, 10$^{th}$ Ed. Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Commission on Life Science, National Research Council*. National Academy Press, Washington, D. C.

[32] Anderson, R. A., and Kozlovsky, A. S. (1985) Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr. 41*, 1177–1183.

[33] National Research Council (2002) *Dietary Reference Intakes for Vitamin A, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A Report of the Panel on Micronutrients, Subcommittee on Upper Reference Levels of Nutrients and of Interpretations and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes*. National Academy of Sciences, Washington, D. C.

[34] Lukaski, H. C. (1999) Chromium as a supplement. *Ann. Rev. Nutr. 19*, 279–302.

[35] Lukaski, H. C. (1987) Methods for the assessment of human body composition: traditional and new. *Am. J. Clin. Nutr. 46*, 537–556.

[36] Ellis, K. J., Bell, S. J., Chertow, G. M., Chumlea, W. C., Know, T. A., Kotler, D. P., Lukaski, H. C., and Schoeller, D. A. (1999). Bioelectrical impedance methods in clinical research: a follow up to the NIH technology assessment conference. *Nutrition 15*, 874–880.

[37] Anderson, R. A., Cheng, N., Bryden, N. A., Polansky, M. M., Cheng, N., Jai, J., and Feng, J. (1997) Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.

[38] Pittler, M. H., Stevinson, C., and Ernst, E. (2003) Chromium picolinate for reducing body weight: meta-analysis of randomized trials. *Int. J. Obesity 27*, 522–529.

[39] Vincent, J. B. (2003) The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent. *Sports Med. 33*, 213–230.

[40] Lukaski, H., Siders, W. A., and Penland, J. G. (in press) Chromium picolinate supplementation in women: Effects on body weight, composition and iron status. *Nutrition*.

[41] United States of America before Federal Trade Commission, Docket No. C-3758, 1997.

*Chapter 5*

# Use of chromium as an animal feed supplement

**Merlin D. Lindemann**

Department of Animal and Food Sciences, University of Kentucky, Lexington, 40546-0215

## INTRODUCTION

The supplementation of chromium in farm livestock is a relatively new phenomenon. While there have been research studies since the 1970s, the actual supplementation of diets has occurred only in the 1990s. Supplementation of diets with minerals that have not been added historically is under the purview of the Food and Drug Administration (FDA) in the United States and the Canadian Food Inspection Agency in Canada. Currently, there are only four forms of chromium that are allowed to be fed to farm livestock in North America and each of those forms has certain restrictions. The allowance of supplementation and the particular restrictions are based upon materials submitted by companies to the appropriate review agency.

The first product allowed to be supplemented as a source of Cr in the United States was chromium picolinate (CrPic). Based upon information submitted related to changes in glucose metabolism when it was supplemented to swine, the FDA stated in 1996 that it would not object to its being marketed as a source of Cr for swine when supplemented at up to 200 ppb Cr in the diet. Following this, information was submitted relative to the effects of chromium propionate (CrProp) on glucose metabolism in swine and the FDA stated in 2000 that it would not object to its being marketed as a source of Cr for swine when supplemented at up to 200 ppb Cr in the diet. Finally, based upon the information submitted relative to the effects of chromium methionine (CrMeth) on non-esterified fatty acid (NEFA) metabolism in swine, the FDA stated in 2003 that it would not object to its being marketed as a source of Cr for swine when supplemented at up to 400 ppb Cr in the diet. The fourth product being supplemented in North America is chromium yeast (CrYst). This form of Cr was approved in Canada in 1999 as a feed additive (i.e., a nutritional additive and not a medicinal additive) for first lactation dairy heifers and is used at the rate of 400 ppb Cr in the diet. Thus, only swine can be supplemented with Cr in the United States and only young dairy cows in Canada, and each of these situations has maximum supplementation limits for the individual product being used as a supplement.

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

The feed industry in North America operates under a variety of controls. Some of those controls may be legislative, others are controls/limits established by governmental agencies (e.g., the level of a drug that can be added may be limited by FDA decisions), while others are limits/guidelines established by industry organizations (e.g., regulations established by the Association of American Feed Control Officials). All of these controls function to provide a feed supply system that is designed to be safe and efficacious for the animals being fed and safe for people consuming products from those animals. These laws, agencies, and guidelines have developed primarily since the 1950s, and that explains why some minerals that have historically been added to diets do not have species limits or inclusion rate limits (e.g., Ca, P, Fe, Zn, Cu, etc. were routinely supplemented to diets before many of these organizations were started), while other minerals (i.e., Se and Cr) that are more recent nutritional discoveries do have limitations and guidelines related to their supplementation.

It should be noted that these four sources of Cr are organic forms of Cr. Some inorganic forms of Cr have been used in livestock research for over a century. For example, chromic oxide is routinely used as an inert marker in digestibility studies. Markers are compounds that are totally indigestible, and that can be used, then, to calculate nutrient digestibility in diets from a small fecal sample; this eliminates the need for total fecal collections (a very laborious and difficult task) for determining nutrient digestibilities and for characterizing the feeding value of different feedstuffs. Additionally, it should be noted that normal diets fed to farm livestock may contain 1000–3000 ppb Cr in the ingredients themselves. And yet, there are distinct and measurable changes in glucose metabolism, sometimes observed with the addition of only 200 ppb of these marketed forms of Cr (a relatively small amount compared to that found naturally in the diet). This oddity of supplementation of very small amounts of a mineral (compared to a mineral like Zn where normal ingredients in swine diets may contribute 25 ppm to the diet and we supplement with a relatively large amount like 50–100 ppm Zn) is totally explained by the bioavailability of the different forms of Cr. Some of the organic forms of Cr are more bioavailable than the inorganic forms previously researched or the natural levels in foods and feeds. Thus, total dietary Cr levels can be very misleading and it is customary to speak simply of supplementation levels.

## FACTORS RELATED TO Cr STATUS OF FARM LIVESTOCK

A general understanding of Cr is necessary to put the research results in context and to determine if there is even a possibility of need in farm livestock. Certain conditions compromise or diminish the Cr status of animals. Two of those conditions are the low availability of Cr from normal feedstuffs and the effect of stress on Cr excretion. Anderson (1987) cites research in humans that indicates that Cr content of many tissues declines with age [1]. The decline is most rapid during the first decade for many tissues (when growth would be most rapid), while for other tissues the decline occurs after the second decade of life. Because of the low availability of Cr from normal dietary ingredients and most inorganic sources, what essentially occurs is that there is very little absorption of Cr and, hence, there is a dilution of body Cr that occurs with growth – being most pronounced in species with the highest relative growth rate. An estimate of

Cr availability for humans from normal dietary ingredients is 0.5% [2] (almost totally unavailable). Bioavailability in farm animals is probably not any greater than that in humans. As an example of relative growth rate, a normal birth weight for a pig is 1.5 kg; that pig will weigh 6 kg at 3 weeks of age (when it is weaned and moved to a nursery), 20 kg at 8–9 weeks of age (when it is moved from the nursery to a grower unit), and 120–130 kg when marketed at about 6 months of age for meat production (thus, an 80-fold increase in body weight in 6 months). As another example of relative growth rate, a broiler chick weighs about 40 g when hatched and will be marketed for production of meat at 6 weeks of age at a bodyweight (BW) of 2.4 kg (a 60-fold increase in 6 weeks). In situations of rapid growth like this, if Cr is a true nutrient need and if Cr bioavailability in natural feedstuffs is low, metabolic problems can develop.

The second condition affecting the Cr status of the animal is stress. Various stressors have been demonstrated to affect Cr excretion and, consequently, Cr status. Some of the stressors reported to increase Cr excretion in humans [3] may also apply to farm livestock. Examples of those are diets with a high glycemic index, lactation, infection, and physical trauma. Other factors that are reported to affect Cr status, or that which place greater pressure on limited Cr supply are parity [4], gestation, and dietary protein level [5]. The most potentially responsive situations in farm animal production, then, to organic Cr supplementation would appear to be those associated with advancing age, with reproduction, and with various production stressors (crowding, transportation, infection, disease challenge, etc.). In general, farm animals are physiologically quite young and geriatric issues would apply only to companion animals (such as dogs, cats, and horses). But for production species, reproductive efficiency and overall animal health related to disease stress or metabolic stress are factors that have major impact on farm profitability; thus, the potential for improvements in well-being and, hence, production related to the supplementation of Cr are of much interest to livestock producers.

Published research related to Cr supplementation of farm animals is more limited than that for humans or laboratory animals. A few articles can be found prior to 1990, but these studies largely evaluated inorganic Cr. The response was, now in retrospect after understanding the low availability of that Cr source, predictably very limited. Since 1990, however, there are a growing number of articles on studies that have evaluated Cr for farm animals and the source is usually an organic source. The majority of these publications are in swine and poultry. A review of Cr in animal nutrition was published in 1997 [6], but those aspects of the review related to actual animal supplementation with organic sources were of research that had primarily been published as abstracts and had not received the scrutiny of peer review. This current discussion will focus on the sources currently being marketed in North America, will examine all major farm species in which they have been evaluated, and will primarily be restricted to sources from peer-reviewed journals that are found in major data bases and selected abstracts that provide both absolute response values and associated statistical evaluations. It should be noted that consistent patterns of response within or across species are more important than is a single study demonstrating a highly significant response that is unsubstantiated by other research studies. Thus, this discussion will also discuss frequency of qualitative responses and patterns of response rather than focusing on selected individual studies.

## SWINE

### Introduction

Swine anatomy and physiology are very similar to the human. This is why swine are often used as models for biomedical research related to human diseases [7, 8] and why they are tissue and pharmaceutical donors for humans (skin grafts, heart valves, insulin, and heparin to name a few items). The normal circulating levels of glucose in pigs are 80–140 mg/dL [9], levels similar to that of humans. One of the human diseases for which swine serve as a model is diabetes. Research in the 1950s [10] demonstrated both that swine vary markedly in their glucose kinetic capabilities (i.e., how hyperglycemic they become after a meal or after a glucose tolerance test and how quickly glucose levels can be reduced back to normal levels) and that the ability of swine to control blood glucose levels decreases as the animal grows. As a result of relatively poor control over glucose homeostasis during glucose tolerance tests, the pig resembles mildly diabetic humans [11]. Because of inherent variation in their capacity to control blood glucose and the declining ability to control that blood glucose as the animal grows (and ages), varying percentages of swine herds will exhibit glucose-control problems. The exact percentage of a herd that is affected will depend also on the amount of "stressors" that would push them toward diabetic-like conditions. Swine are not known to have a situation similar to Type 1 diabetes in humans where the body does not produce insulin. If that condition ever existed, it would have eventually been removed from the swine population because those pigs would have died early in life in the absence of insulin, and the associated blood glucose problems would not have reproduced. However, the problems observed in swine are similar to Type 2 diabetic-like conditions where blood glucose levels rise in the presence of insulin, the tissues become increasingly resistant to the insulin, and, then, health problems related to blood glucose may occur.

In addition to the anatomical and physiological similarities with humans, there are some dietary similarities. The diets of pigs yield primarily glucose upon digestion. The diets are cereal-grain-based diets being composed of 60–85% grain depending on the age of the pig and how much protein, vitamin, and mineral supplements are required. These grains are primarily corn and sorghum in the United States and corn, wheat, and barley in Canada. While cereal grains contribute many nutrients to the diet, they are primarily an energy source. The energy is in the form of starch and, thus, the end product of digestion is glucose. The rate of digestion and the consequent absorption of glucose will depend on a variety of factors such as meal size, particle size of the ground grain, type of starch in the grain, and age of the pig.

For these reasons pigs are one of the most likely farm livestock species to potentially benefit from Cr supplementation. The research with pigs in the 1990s resulted in its being the first North American farm species that could have Cr supplemented to the diet.

### Metabolic effects

Amoikon et al. evaluated Cr supplementation effects on glucose metabolism with the classic methodologies of intravenous Glucose Tolerance Tests (IVGTT) and Insulin Challenge Tests (IVICT) [12]. Glucose disappearance rate was increased and glucose half-life was decreased in pigs fed 200 ppb Cr from CrPic ($P < 0.04$), thus demonstrating

an improvement in tissue insulin sensitivity consistent with the improved insulin efficiency noted in mature female swine in the first reported reproductive study [13]. Additional research with growing pigs using IVGTT and IVICT that has shown Cr-related changes in glucose or insulin responses has been published for CrPic [14], CrYst [15], CrProp [14], and CrMeth [16].

The positive effects of Cr supplementation from CrPic on insulin sensitivity of growing pigs had also been demonstrated in settings that did not utilize the IVGTT and IVICT. Evock-Clover et al. examined the potential interaction of Cr supplementation and porcine somatotropin administration in growing pigs [17]. Porcine somatotropin administration is known to result in alterations in glucose and insulin metabolism; and this known effect of somatotropin was evident as anticipated. Supplementation of the diet with Cr resulted in lower serum insulin and glucose concentrations relative to the controls ($P < 0.05$) and it served to normalize the aberrant increases in glucose and insulin resulting from the somatotropin administration. A response of improved insulin efficiency after consumption of a normal meal in pregnant gilts (first parity females) was also demonstrated by work at the University of Missouri with CrPic [18].

**Chromium for weanling pigs**

The predominant practice in North America is to wean pigs at an age that ranges from 17 to 30 days depending on the individual farm. These weanling pigs are moved away from the sow (female swine are called "sows" after they have given birth to their first litter) to a nursery where they are fed and housed for a period of 4–6 weeks. These young pigs go through a variety of changes that could be considered stressful. From a social perspective, the actual weaning can be considered psychologically stressful as they are removed from the sow and perhaps regrouped with other pigs. Regrouping of any animal is accompanied by physical bouts to establish the dominance structure within the animal pen. From a dietary perspective, the diet changes from a liquid diet (milk) to a dry diet. The principle energy sources in the liquid diet are lactose (a disaccharide comprised of glucose and galactose) and milk fat; the principle energy source in the dry diet is starch. This change in form and composition of diet can be accompanied by a period of low feed intake and decreased digestibility as the pig adapts to the diet texture and as it adapts to a need for different digestive enzymes to digest the different amount and source of nutrients in the diet. The combination of changes makes this a stressful time of transition, and there is interest in any nutritional modification that might provide greater health and well-being.

Additionally, newborn pigs receive a tremendous amount of immunoglobulins from the colostrum of the sow. Based on the half-life of these immunoglobulins and the development of the pig's own active immunity, there is a window of time around 3–5 weeks of age when the young pig may be immunocompromised and susceptible to disease challenge. The fact that this immunological adaptation occurs when all of the other stressors are occurring is unfortunate. Some of the early research with beef cattle indicated a possible improvement in immunocompetence as a result of Cr supplementation (to be discussed later). Thus, while the research with weanling pigs is not extensive, the majority of studies evaluate some aspect of immunocompetence.

A series of four studies was conducted at the National Chai-yi Institute of Technology in Taiwan to investigate the relationship between Cr and immunity. In the initial

study [19], a limited number of pigs (n = 24) weaned at 4 weeks of age were fed diets with 0, 200, 400, and 800 ppb Cr from CrPic for a period of 7 weeks. Pigs were vaccinated at 2 and 5 weeks post-weaning with a virus naturally found in some pig herds. There were no clear effects of Cr supplementation on the normal growth performance measures (average daily feed intake, average daily weight gain, and feed efficiency [the ratio of daily feed intake to daily gain]), but it appeared that the 400 ppb Cr may have improved daily gain in the first 4 weeks after weaning. The Cr supplementation increased primary and secondary antigen-specific titers, tended to increase IgG at week 6 and IgM throughout the study, and tended to increase phytohemagluttinin (PHA)-induced peripheral blood mononuclear cell blastogenesis early in the weaning period. A similar treatment structure was subsequently used with specific-pathogen-free pigs (pigs born and raised in a way that they have not been exposed to certain pathogens to which pigs are normally exposed) to examine the effects of Cr on lymphocyte subsets [20]. There were no clear Cr effects, but there were some indications that 400 ppb Cr changed the lymphocyte population distribution at some times. The level of 400 ppb Cr from CrPic was then used in two larger studies. The first of these two studies evaluated the response of pigs to supplementation and to injection with lipopolysaccharide (LPS, an endotoxin that will elicit a fever response characterized by reduced feed intake, elevated body temperature, and an increase in plasma cortisol and tumor necrosis factor-alpha) [21]. The supplemental Cr did not influence the physiological response of the pigs to the endotoxin injection. In the second study [22], pigs were vaccinated with a natural antigen at 8 weeks of age (Experiment 1) or at 3 and 8 weeks of age (Experiment 2). Supplemental Cr did reduce rectal temperature of pigs following the 8-week injection, but it did not affect any growth measure, plasma immunoglobulin levels, humoral antigen-specific titers or cell-mediated immune response as measured by skinfold swelling to an intradermal injection of PHA.

Neither van Heugten and Spears [23], who evaluated 200 ppb supplemental Cr from CrPic, nor Tang et al. [24], who evaluated 200 ppb Cr from either CrPic or CrYst, observed any meaningful improvement in immune response to LPS injections, antibody titer to either natural or novel antigens, or skinfold swelling test. However, Lee et al. reported that supplementation with 400 ppb Cr from CrPic could modulate aspects of the immune response to LPS injection in the weanling pig [25, 26]. Tang et al. did report that CrYst increased daily gain as a result of slight increases in feed intake in those pigs fed CrYst [24].

A slightly different approach was used by van de Ligt et al. who attempted to evaluate longer-term Cr status rather than just short-term Cr supplementation [27, 28]. Female swine were fed 200 ppb Cr from CrPic throughout growth and development and through two parities, and the immune status of their piglets was evaluated. Females were injected with a novel antigen prior to giving birth to the pigs, and general and specific immunoglobulin responses were measured in sow serum, milk, and piglet serum. The investigators found that supplementation of the dam had minimal effects on humoral antibody response of the dam or its transfer to the neonate. A subset of the pigs was then followed post-weaning for a period of 6 weeks (pigs were continued on the same supplementation regime as their dams) with the observation that the supplemental Cr did not affect growth performance or immune status.

In summary, in spite of a few positive observations, there do not appear to be any generalized improvements in immunocompetence in young pigs with the supplementation of Cr, even though this is a period of time when the immune system of the pig is compromised. Further, while there are two studies that report improved growth and improved nutrient digestibility with supplementation of 200 ppb Cr from CrPic [29, 30], there does not appear to be any generalized improvement in growth performance during this period with Cr supplementation. It should be noted that young pigs such as used in these studies would have been only 5–20 kg BW (compared to a mature body size of 150–250 kg BW) and would not have experienced very much of the normal dilution of body Cr that occurs with age (as presented earlier) and thus may not be in particular need of the nutrient.

**Chromium for growing pigs**

Following the nursery phase, pigs are generally moved into another building in which they are fed until they reach market weight. In the early part of the twentieth century, pigs were extremely fat because the fat had commercial value as it was used to make nitroglycerin which had military uses [31]. Following World War II, this need for fat ceased, and a consuming public was more interested in a leaner product. Thus throughout the 1960s, 1970s, and 1980s, there was an intensive effort to change the body type of the pig to be more lean through genetic selection and through dietary modifications that would result in more lean (muscle) deposition and less fat deposition. In the current marketing structure, producers are rewarded for pigs with higher amounts of muscle and are discounted for pigs with high fat content. The interest in Cr supplementation during this period in the life of the pig is primarily for its potential impact on muscling, and secondarily for any potential improvement in feed efficiency (if a pig can gain the same amount of weight on less feed, there is an obvious reduction in cost for the production of that pig). Muscling in pigs is generally measured by the cross-sectional area of the longissimus muscle (a large muscle that lays perpendicular to the backbone and is processed into the "pork chop"), the fat depth at the 10th rib (this would equate to the amount of fat on the outside of the pork chop), or by the overall lean percentage in the carcass which is computed from the weight of the pig, the longissimus muscle area (LMA), and the 10th rib fat depth (TRF).

Great enthusiasm for the potential of Cr to improve muscling was generated by the initial reports of a series of trials with CrPic from the laboratory at Louisiana State University (LSU) [32]. Researchers reported increased LMA and decreased TRF of carcasses at market when Cr was fed to the growing pigs. In some of the trials the LMA increased more than 20% and the TRF decreased more than 25%. Interestingly, in the three trials reported, the magnitude of increase in LMA and decrease in TRF was greater as the starting weight of the pigs on test decreased from 37.8 kg in Trial 1 to 22.4 kg in Trial 3. This effect is not surprising because any benefit that Cr might have on carcass measurements would be cumulative, so that number of days of supplementation should be related to the desired response. These researchers also established that supplementation of Cr as chromium chloride or of picolinic acid in the same quantity as that supplied when 200 ppb Cr was supplied as the specific compound CrPic did not elicit any positive muscling effects. The lack of effect of Cr as chromium chloride was consistent with previous evaluations [33]. Studies at Virginia Polytechnic Institute and State University

(VT) [13] corroborated the results observed at LSU. In Trial 1 at VT, when the starting weight of the pigs was 40.9 kg, there were no improvements in muscling, but in Trial 2, when the starting weight of the pigs was 14.5 kg, improvements ($P < 0.05$) in both TRF and LMA were noted. Additionally, results from Trial 2 showed a clear improvement in feed efficiency when Cr was supplemented to one of the diet types. No consistent effects of Cr supplementation were noted on growth rate in the trials at LSU or VT.

The results of supplementing Cr since those initial positive studies have not been consistent. Lindemann summarized 20 trial comparisons related to feeding of 200 ppb Cr from CrPic (17 of which were from North American research) [34]. Examination of some of the aspects of the trial methodology revealed that the trials that demonstrated the greatest responses were those that were formulated to the recommended protein requirement estimates [35] while those studies that employed diets formulated in excess of requirement estimates did not see a very large response. In fact if the studies were grouped into those in which excess protein was fed and those in which recommended protein was fed, the mean feed/gain values (units of feed consumed/units of weight gain) in the excess-protein studies were 2.96 vs. 2.95 with supplemental Cr, while in the recommended-protein studies the mean feed/gain values were 3.02 vs. 2.81 with supplemental Cr. Thus, there is apparently no need to reformulate to a higher protein level when Cr is supplemented in an effort to provide more amino acids for potential protein deposition. There is apparently an increase in digestibility of the diet that allows more nutrients to be absorbed or there is an increased efficiency of use of the absorbed nutrients.

Lindemann also reviewed the reported carcass effects from those trial comparisons [34] and found a mean increase in LMA of 2.7 $cm^2$ (about 7%; with 13 numerically positive responses out of 15 comparisons) and a mean reduction in TRF of 3.3 mm (about 13%; again 13 positive responses out of 15 comparisons). In very few of the individual trial comparisons were the responses statistically significant. Studies with farm animals are difficult due to relatively high population variability. This variability is higher than that of some studies with laboratory animal species that may use cloned animals or animals of extremely similar genetics. In light of this difficulty, evaluations of groups of studies are useful to determine frequency of response. The results of this type of evaluation with carcass measurements would suggest that the response may be real (13 out of 15 positive responses for each measure), but the effects are not overly large.

A summary of studies made available since the Lindemann (1999) review is provided in Table 1. It can be seen that there is not a consistent pattern in the response with 14 positive responses (i.e., a reduction in feed/gain) out of 23 possible comparisons to the control value, and there were both statistically significant improvements and detriments noted. Dividing the responses into excess vs. recommended protein in these studies was not possible because of several factors related to protein deposition relative to protein need in newer genetic lines of pigs. As stated with weanling pigs, if there are improvements in feed efficiency, they would have to be mediated either by improved digestibility of the diet or by improved efficiency of nutrient metabolism. An improved digestibility with CrPic supplementation has been demonstrated [36]. A set of four nutrient retention experiments were conducted to assess the effect of feeding 200 ppb Cr as CrPic on dry matter digestibility and nitrogen balance of growing pigs. Percentage

Table 1
Effect of supplemental chromium on feed/gain of pigs[a]

| Source | Chromium (ppb) | | | Reference |
|---|---|---|---|---|
| | 0 | 200 | 400 | |
| CrPic | 3.14 | 3.15 | 3.46 | Liarn et al., 1993 [37] |
| CrPic[b] | 3.18 | 3.37* | 3.15 | Lien et al., 1996 [38] |
| CrPic | 3.24 | 3.23 | 3.16 | Min et al., 1997 [39] |
| CrPic | 3.82 | | 3.65 | Ward et al., 1997 [40] |
| CrPic | 3.36 | | 3.50 | Ward et al., 1997 [40] |
| CrPic[c] | 3.42 | 3.44 | | Gundel et al., 1998 [41] |
| CrPic | 3.44 | 3.44 | | Lien et al., 1998 [42] |
| CrPic | 3.26 | 3.27 | | Hanczakowska et al., 1999 [43] |
| CrPic | 3.51 | 3.44 | 3.44 | Lien et al., 2001 [44] |
| CrPic | 3.24 | 3.09 | | Urbańczyk et al., 2001 [45] |
| CrProp[d] | 2.86 | 2.82 | | Matthews et al., 2003 [46] |
| CrProp[d] | 3.45 | 3.33 | | Shelton et al., 2003 [47] |
| CrProp | 3.72 | 3.62 | | Matthews et al., 2005 [48] |
| CrYst | 2.88 | 2.75 | | Simek et al., 1996 [49] |
| CrYst | 3.26 | 3.42* | | Hanczakowska et al., 1999 [43] |
| CrYst | 2.78 | 2.61* | 2.73 | Lemme et al., 1999 [50] |
| CrYst[e] | 3.00 | 3.13 | | Lemme et al., 2000 [51] |
| CrYst[f] | 2.94 | 2.89 | | Lemme et al., 2000 [51] |

[a]Studies listed are those in which pigs were given ad libitum (or near ad libitum) access to feed and which were not listed in Lindemann (1999). Statistically significant improvements in an individual trial of a supplemental value from the control is noted by an * ($P < 0.05$).
[b]Highest level supplemented was 600 ppb.
[c]Supplemented level was 230 ppb.
[d]Data are pooled across two dietary energy levels.
[e]Diet was based on barley to simulate a low glycemic index diet.
[f]Diet was based on wheat to simulate a high glycemic index diet.

nitrogen absorption, percentage nitrogen retention, and dry matter digestibility were all increased ($P < 0.01$–$0.07$) by feeding Cr. Thus, while the results of improved feed efficiency are not observed in all studies, they are plausible given these demonstrated effects on digestibility. If there is a true effect of a biologically available Cr on feed/gain, then factors related to dietary nutrient density, genetics of the pigs fed, or other factors not yet identified may explain when positive effects would be expected to occur and when they would not be expected.

Effects on carcass measures since the Lindemann (1999) review are illustrated in Table 2. In this comparison of published reports, 14 of the 17 comparisons for effect on LMA result in an improvement (an increase) while for TRF 19 of the 23 comparisons result in an improvement (a decreased value). None of the three forms listed give uniformly positive responses. When statistical significance was noted in individual trials, it was only of improvements, and there were no significant detriments noted. Of these

Table 2
Effect of chromium supplementation on carcass measurements[a]

| Source | LMA ($cm^2$) | | | TRF (cm) | | | Reference |
|---|---|---|---|---|---|---|---|
| | Chromium (ppb) | | | Chromium (ppb) | | | |
| | 0 | 200 | 400 | 0 | 200 | 400 | |
| CrPic | | | | 2.50 | 2.09 | 1.85* | Liarn et al., 1993 [37] |
| CrPic[b] | 42.4 | 46.4* | 59.7* | 1.75 | 1.46* | 0.88* | Lien et al., 1996 [38] |
| CrPic | 22.8 | 24.3 | 23.5 | 2.64 | 2.22 | 2.34 | Min et al., 1997 [39] |
| CrPic | 30.9 | | 31.1 | 2.85 | | 2.80 | Ward et al., 1997 [40] |
| CrPic | 35.4 | | 34.6 | 2.80 | | 2.40* | Ward et al., 1997 [40] |
| CrPic | 55.0 | 61.7* | | 3.86 | 3.49* | | Lien et al., 1998 [42] |
| CrPic | 28.6 | 31.4* | | 3.14 | 2.95* | | Renteria and Cuaron, 1998 [52] |
| CrPic | 50.3 | 52.7 | | 2.82 | 2.77 | | Hanczakowska et al., 1999 [43] |
| CrPic | 46.6 | 54.2* | 56.2* | 3.43 | 2.95* | 2.96* | Lien et al., 2001 [44] |
| CrPic | 50.9 | 55.5* | | 2.90 | 2.84 | | Urbańczyk et al., 2001 [45] |
| CrProp[c] | 37.5 | 37.1 | | 2.20 | 2.22 | | Matthews et al., 2003 [46] |
| CrProp[c,d] | 45.8 | 46.6 | | 1.72 | 1.60 | | Shelton et al., 2003 [47] |
| CrProp | 46.7 | 44.7 | | 1.54 | 1.49 | | Matthews et al., 2005 [48] |
| CrYst | 41.2 | 43.3 | | 1.60 | 1.42 | | Simek et al., 1996 [49] |
| CrYst | 50.3 | 51.3 | | 2.82 | 2.86 | | Hanczakowska et al., 1999 [43] |
| CrYst | | | | 1.79 | 1.88 | 1.77 | Lemme et al., 1999 [50] |
| CrYst[e] | | | | 1.86 | 1.76 | | Lemme et al., 2000 [51] |
| CrYst[f] | | | | 1.86 | 1.87 | | Lemme et al., 2000 [51] |

[a] Studies listed are those in which pigs were given ad libitum (or near ad libitum) access to feed and which were not listed in Lindemann (1999) [34]. Statistically significant improvements in an individual trial of a supplemental value from the control is noted by an * ($P < 0.05$).
[b] Highest level supplemented was 600 ppb.
[c] Data are pooled across two dietary energy levels.
[d] Response for LMA was present in the normal energy diet but not in the low energy diet.
[e] Diet was based on barley to simulate a low glycemic index diet.
[f] Diet was based on wheat to simulate a high glycemic index diet.

two measured carcass values, there appears to be more of an effect on TRF. The high frequency of positive responses would indicate that the effects may be real. Some of the studies have evaluated pork quality by various measures in addition to pork quantity (as implicated by increased LMA) and have reported positive effects [46, 47, 48].

**Chromium for reproducing pigs**

A critical physiological stressor common to both humans and pigs is pregnancy. A condition familiar to many is gestational diabetes (also referred to as pregnancy-induced diabetes) wherein a woman may develop glucose control problems during pregnancy that return to normal after the child is born. Research with women who were pregnant demonstrated that hair chromium content (an indicator of body chromium status) of first-pregnancy women was about three-fold that of women having other-than-the-first child [4]; pregnancy greatly diminishes maternal body chromium stores and takes time to replenish. Thus, frequent pregnancies diminish a woman's Cr status and may predispose her to chromium-related glucose kinetic problems during pregnancy. In normal swine production, sows are often re-bred at the first observed estrus after weaning (a period of only 4–9 days post-weaning if the sow is in good physical condition) resulting in few days to replenish body stores of Cr. And, indeed, in pigs there are glucose-control problems associated with pregnancy. Research with sows at Oregon State University in the 1970s resulted in the conclusion that "pregnancy in the sow is a diabetogenic event" [53]. They further illustrated that the problem becomes worse in the latter stages of pregnancy. A study from the Netherlands [54] evaluated blood glucose levels after a meal in sows that were in late gestation, and the researchers observed that sows that had the lowest ability to control blood glucose (i.e., the blood glucose levels rose higher after eating the meal and took longer to return to baseline) had the highest incidence of mortality in the newborn piglets during the first 7 days after birth. This is consistent with an increased mortality of infants born to women with gestational diabetes. In fact, Bouillon-Hausman et al. concluded with regard to gestational diabetes in humans that "the pig appears to be an appropriate animal model for studying etiology of gestational diabetes and for further characterizing effects of diabetic pregnancies on pre- and post-natal growth and development" [55]. Thus, while the physiological similarities allow a cross-species understanding of glucose, diabetes, and pregnancy, it may also allow a cross-species understanding of some of the potential effects of Cr supplementation.

So, sows are the individuals within the swine species most subject to glucose-control problems because of two factors. First, swine in general have glucose-control problems as they age, and secondly, pregnant swine are subject to gestational diabetes. Within any particular herd, then, the swine most subject to glucose-control problems would be the reproducing swine. And, then, by extension, the swine most likely to respond in a positive manner to Cr supplementation would be these same animals. Because reproductive efficiency is a primary factor determining the profitability of swine production units, nutrition of the sow is an area of much commercial interest.

Table 3 lists the reported studies of Cr supplementation to reproducing pigs. It is difficult to observe statistically significant effects in sow research because of extremely high variability in many reproductive measures. It is estimated, based on the variability for litter size, that 114 sows per treatment are needed to detect a 10% difference in

Table 3
Effect of supplemental chromium on number of live pigs born/litter[a]

| Source | Litter size | | | Reference |
|---|---|---|---|---|
| | Chromium (ppb) | | | |
| | 0 | 200 | >200 | |
| CrPic[b] | 8.93 | 11.25*[a] | | Lindemann et al., 1995a [13] |
| CrPic[c] | 9.60 | 10.50 | | Lindemann et al., 1995b [57] |
| CrPic[d] | 9.94 | 10.01 | | Campbell, 1998; Trial 1 [58] |
| CrPic[e] | 11.41 | 11.54 | | Campbell, 1998; Trial 2 [58] |
| CrPic[f] | 9.55 | 10.60 | | Charraga and Cuaron, 1998 [59] |
| CrPic[g] | 10.05 | 10.42* | | Hagen et al., 2000 [60] |
| CrPic[h] | 10.77 | 11.45* | | Lindemann et al., 2000 [61] |
| CrPic[i] | 9.49 | 9.82 | 10.94* | Lindemann et al., 2004 [62] |
| CrMet[j] | 9.60 | 11.00* | 10.90* | Perez-Mendoza et al., 2003 [63] |

[a]Statistical significance in an individual trial is denoted by an * ($P < 0.05$).
[b]Females supplemented since 4 weeks of age; study conducted for two parities.
[c]Females received supplement about 1 week before breeding; study conducted for three parities.
[d]Females received supplement the day after breeding; study conducted with females of different parities with results pooled across parity.
[e]Females received supplement for 35 days after breeding and no supplement for the remaining 80 days of gestation.
[f]Females received supplement for 3 weeks prior to mating; all females were first parity.
[g]Females received supplement 6 months prior to beginning treatment evaluations on the farms; a total of 48 000 sows used on the study.
[h]Females received supplement about 4–7 days before breeding and were fed for as many as three successive parities.
[i]Females supplemented after breeding; study conducted for a minimum of two parities; multiple doses used in the study; the higher dose listed was 600 ppb Cr.
[j]Females supplemented during the previous lactation and until rebreeding; no supplementation during gestation; multiple doses used in the study; the higher dose listed was 400 ppb Cr.

litter size at birth at an 80% success rate and at a 5% probability level [56]. To use this number of sows is not possible for most university research facilities. Thus true biological effects that may have much economic impact may be overlooked simply because of low numbers of experimental units. Despite this limitation of much of the sow research, statistically clear increases in the number of pigs born alive for each litter are seen frequently in Cr research. An interesting observation of the results from Table 3 is that all responses published have been numerically positive. There is the danger that negative responses may not be submitted or accepted for publication, but the pattern of response here in these studies, even in those not noted as statistically significant, is very clear. The first reported study had an extremely large response and is the only study

in which the females were fed through all of the growing/developing stage. The second reproductive study did not have as large a response, but an interesting observation of that work is related to the length of supplementation. In that study, the gilts did not receive supplemental Cr until the breeding started for the first parity. The response was smallest in parity 1 (0.4 pig/litter) but increased through parity 3 (0.8 pig/litter in parity 2 and 2.0 pig/litter in parity 3). The litter size response then after a period of supplementation was similar to that observed in the first study when animals were supplemented for a long time prior to breeding. Time/dose relationships regarding supplementation certainly may be responsible for varying magnitude of responses across reported trials.

The largest study reported was conducted in a commercial setting with 48 000 sows and resulted in about 100 000 litters for a total of over a million piglets on the study [60]. Results were smaller than in some of the university studies but were statistically significant and are probably more realistic in terms of the total industry impact of Cr supplementation. It demonstrated increases in live born pigs (0.37 pigs/litter; $P < 0.02$) that were a combination of an increase in total piglets born (0.22), and a decrease in stillborn piglets (0.05) and mummified piglets (0.10). The numerical decrease in stillborn and mummified pigs is presumably related to late-term uterine conditions that affect fetal survival while the increase in total born is probably somewhat a function of ovulation rate [64].

Some of the studies supplemented for only very short periods of time either before breeding or immediately after breeding and the results are also numerically positive. If time/dose relationships are indeed factors in supplementation (and they should be if Cr is like other nutrients), then not only is the length of supplementation an issue of concern but the level of supplementation is an important consideration. Because muscle is a target tissue for Cr and constitutes the single largest body tissue, examination of Cr intake per unit BW may be an appropriate evaluation of supplementation adequacy. Depending on the degree of depletion of Cr reserves in the body and the priority of different tissues for absorbed Cr, it may take a period of time before reproductive tissues receive an amount of Cr necessary to maximize their function. This concept was used by a regional research group [62] who calculated the amount of Cr received by growing animals in studies that had evaluated responses in IVGTTs and IVICTs. The value they computed was about 7.5 μg Cr/kg BW/day. When this value is extended to reproducing animals (based on their size and feed intake), it would take about 500–600 ppb of supplemental Cr in the diet to supply an equivalent amount per unit BW to that received by growing animals. The study they then conducted used multiple levels of supplemental Cr from CrPic (0, 200, 600, and 1000 ppb) for a minimum of two parities. They observed a quadratic response to Cr supplementation that was highest at 600 ppb of supplementation.

In addition to these time/dose relationships, the magnitude of response appears to be dependent on the performance of the control animals. In situations where performance is good, the response is less than when performance is not as good. Perhaps it is better stated that when performance is compromised for a variety of reasons, there is more potential for responses from nutritional supplementation; alternately, a portion of diminished performance can be associated with a diminished Cr status that, when restored, allows a fuller expression of an animal's genetic potential.

In the first reported reproductive study involving Cr supplementation, blood samples taken in mid-gestation demonstrated very clear differences in tissue sensitivity to

insulin [13]; all sows had similar serum glucose values pre-feeding and 2 hours post-feeding, but the 2-hour insulin levels were three-fold higher in unsupplemented sows ($P = 0.01$). This response to Cr would be consistent with the response observed in gestational diabetic women wherein Cr supplementation elicited more pronounced effects on insulin measures than on glucose measures [65]. Garcia et al. confirmed these effects by demonstrating dose-related effects of supplemental Cr on tissue sensitivity to insulin in pregnant gilts as illustrated by a reduced Insulin/Glucose ratio in the blood 2-hours post-feeding [18]. In further evaluations of post-feeding responses in pregnant sows supplemented with 200 ppb Cr from CrPic, Woodworth et al. demonstrated that the post-feeding glucose peak of supplemented sows was lower than that of unsupplemented sows, and the mean glucose concentration (over a broader time frame) was lower in the supplemented sows [66]. Additionally, the mean insulin concentration was lowest for sows supplemented with CrPic and highest for unsupplemented sows.

It should be noted that in situations of compromised tissue sensitivity to insulin, there are two means whereby more of an "insulin effect" can be accomplished. Those two means are, first, to physically inject insulin (as is done by human diabetics; this effectively pushes the system to provide more of an insulin effect) and, secondly, to restore tissue sensitivity to insulin (thereby allowing endogenous insulin to accomplish more of an effect). Thus, improved tissue sensitivity (which can be accomplished by Cr supplementation) and insulin injection can effectively provide some similar responses and are in some senses analogous. Thus, research in which exogenous insulin has been administered and has demonstrated an effect can be examined for a fuller understanding (or for confirmation) of the potential effects of supplemental Cr.

Indeed, exogenous insulin has been demonstrated to increase the frequency of luteinizing hormone (LH) pulses in restricted-fed gilts and has been reported to increase ovulation rate of gilts [67]. A series of research reports from Mississippi State University (MSU) has further illustrated the effects of insulin administration on swine reproduction. Cox et al. initially demonstrated that insulin injection of gilts immediately preceding estrus increases ovulation rate [68]. This is consistent with research with 200 ppb Cr from CrPic conducted in Brazil [64] that demonstrated a clear increase in viable embryos at a mid-gestational slaughter (14.0 vs 12.7, $P = 0.05$; analogous to mid-gestation litter size) with the mechanism being a combination of ovulation rate (17.1 vs 16.5) and embryo survival (81.9% vs 77.0%). The MSU research group has also noted that insulin injection for 5 days after weaning to primiparous sows increased follicular estradiol and progesterone levels [69]. The observation of elevated progesterone levels is consistent with that of Garcia et al., who, while demonstrating the dose-related effects of supplemental Cr on tissue sensitivity to insulin in pregnant gilts, added the observation that oxytocin and serum progesterone were also affected by CrPic [18]. The MSU group further demonstrated that the effects of that short-term insulin administration after weaning and before breeding could still be seen at the next farrowing where increases in litter size of up to one pig/litter were observed with some of the insulin treatments [70]. They also demonstrated that the insulin administration effects for that 4- or 5-day period could be dependent on the metabolic or nutritional state of the animal and could increase litter size by as much as two pigs [71]. Clearly, linkages between supplemental Cr, glucose, and insulin exist [12, 13]. Further, effects of glucose levels and metabolism on reproductive performance have been known for decades [10, 53], and effects of insulin on

reproductive performance have been recently demonstrated (by the MSU group). Thus, the improvements in litter size associated with Cr supplementation in the studies of the 1990s are consistent with the results of the studies examining direct injection of insulin. While Cr may have other metabolic impacts on pigs, the effects on insulin/glucose relationships and their consequent metabolic effects are undoubtedly a primary mechanism by which its effects on reproduction are exerted.

**Summary for swine**

Supplementation of bioavailable sources of Cr to swine is not uniformly efficacious (or, is not a matter of routine need). Supplementation of young pigs immediately after weaning has not been demonstrated to have any uniform effect on either growth measures or on immunocompetence. Supplementation of the growing animal has provided some responses of improved feed efficiency and improved muscling that are attractive, but the responses have not been uniform in magnitude which suggests a situational dependence for supplementation or other factors (e.g., dietary, environmental, and management) that have not been identified that control when responses are observed. In reproducing sows, the responses are uniform, and that response is of increased prolificacy. The magnitude of the response has also varied here but appears to be related to length of supplementation, dose of supplementation, and perhaps body size of the animal. The fact that responses to supplementation are minimal in the very young pig, occasionally present in the growing/developing pig, and uniformly present in the mature pig suggests a changing Cr status with age. Thus, a lower level of supplementation of the young may avoid a declining Cr status with age and result in a lower supplementation need in adults.

## CATTLE

**Introduction**

Cattle are different than swine in that cattle generally consume a high forage diet (although some cattle may be fed a diet with higher grain content for a brief period of time prior to processing for meat). This high forage diet is fermented in the first compartment of the stomach (the stomach has four compartments in cattle), and the carbohydrates from grain or from forage are absorbed as short-chain volatile fatty acids (these are the primary energy-yielding compounds absorbed from carbohydrate fermentation in the gastrointestinal tract rather than glucose as in pigs and people). Cattle do have a physiological need for glucose which they can manufacture from one of the volatile fatty acids, and they do absorb a small amount of glucose in the small intestine from carbohydrate that escapes fermentation. Cattle will generally have blood glucose levels about half that which are observed in pigs and people (42–75 mg/dL [72]). Thus, if the only effects of Cr supplementation are on glucose and insulin, cattle might be expected to have little or no response to supplementation.

**Metabolic effects**

Despite the possibly low response to supplemental Cr, researchers at LSU demonstrated in 1994 that supplementation of 370 ppb Cr from CrPic to growing dairy calves increased glucose clearance rates after glucose infusion in two IVGTTs as well as during IVICTs [73]. But, in spite of these clear effects on glucose metabolism, there were

no observed changes in growth performance related to supplementation for more than 10 weeks. Using younger calves supplemented with 1000 ppb Cr from birth to 8 weeks of age, Depew et al. failed to observe supplementation effects on glucose and insulin measures but did observe lower NEFA levels; again, effects on growth performance were inconsistent [74]. Kegley et al. evaluated the effects of 400 and 800 ppb Cr from CrMeth in growing beef calves following 3 weeks of supplementation and observed a quadratic increase in insulin levels following an IVGTT and a linear increase in glucose clearance rate in response to an IVICT [75].

**Chromium for young calves**

Because of the aforementioned relationship of Cr and stress, much of the early cattle work was with cattle that were stressed in a variety of manners that might, theoretically, increase the Cr requirement or increase the Cr loss that would then precipitate a situation that was responsive to supplementation. A series of trials was conducted at the University of Guelph evaluating this possibility. Chang and Mowat examined the supplementation of Cr to calves that had experienced the stressors associated with placement of calves from a situation of grazing on rangeland to being fed in a feedlot (stressors would be inter-province transport, crowding during transport, potential novel antigen exposure from other calves, limitation of feed and water supply during the period of transport) [76]. Upon arrival at the feedlot, half of the calves were given an antibiotic injection as a potential ameliorator of stress effects, and each of those groups was further divided into supplementation with Cr from a high-CrYst at the rate of 0 or 400 ppb until day 28 after arrival in the feedlot. These first 28–30 days can sometimes be associated with depressed performance depending on the degree of "shipping stress" the calves have experienced. Improvements ($P < 0.05$) in growth rate of 30% and in feed efficiency of 27% were observed in the first 28 days after arrival in calves supplemented with Cr that had not been injected with a long-acting antibiotic; no performance improvement was observed due to Cr supplementation in calves that had been injected with a long-acting antibiotic. For the next 70 days, the Cr level was reduced to 200 ppb and an additional dietary factor (protein source) was introduced. While there were no performance benefits in the 70-day period that followed, the supplementation of Cr did reduce serum cortisol ($P < 0.01$) and increase ($P < 0.05$) serum IgM and total immunoglobulins in calves fed one of the two protein sources. Another trial with multiple levels of Cr (0, 200, 500, or 1000 ppb) from high-CrYst demonstrated that the highest level of supplementation increased ($P < 0.05$) growth rate by 29% and feed intake by 15% compared to unsupplemented animals during the first 30 days in the feedlot [77]. There also appeared to be a reduction in morbidity (sick calves that needed treatment) with all Cr supplementation levels requiring fewer treatments, but the differences were not statistically significant; however, rectal temperatures on all Cr treatments were lower ($P < 0.05$) on day 2 and day 5 post-arrival at the feedlot while serum cortisol levels at day 28 were decreased with increasing levels of supplementation (69.4, 63.2, 58.6, and 47.3 mol/L; $P < 0.05$) which revealed a relationship between Cr and these indicators of stress and well-being. A third trial compared two sources of Cr (high-CrYst and an amino acid chelated Cr) for the initial 35 days after receipt into the feedlot [78]. High-CrYst tended to reduce morbidity, but the chelated Cr was more effective resulting in morbidity only 1/3 that of the unsupplemented controls ($P < 0.05$). Growth rate was 41% higher during the first

21 days with chelated Cr ($P < 0.05$), primarily due to the reduction in morbidity; for the total 35-day trial, feed efficiency was improved with the high-CrYst ($P < 0.05$) relative to the unsupplemented controls with the chelated Cr having an intermediate feed efficiency. With regard to the effects of Cr supplementation on day 34 cortisol levels, the values for the two Cr sources were actually greater than that of the unsupplemented treatment. A final trial with stressed feeder calves in which CrYst was supplemented at the rate of 750 ppb Cr demonstrated no effects on performance or morbidity although serum cortisol values were reduced in Cr-supplemented calves 55 days after initiation of the trial [79]. These last two trials, where little response was observed, were conducted with calves purchased in local sales in Ontario that would not have been transported as great a distance as those in the initial two studies in which the Cr effects were more pronounced. This series of studies is summarized nicely by Fig. 1 where Dr Mowat pools the early growth performance of all calves in these studies and groups it into that of the poorest, intermediate, and best performing groups [80]. The supplementation of Cr is of most benefit in the groups of calves expressing the lowest performance which is presumably related to the greatest cumulative degree of stress (social, dietary, immunological, etc.) that they have experienced. The potential benefit of supplemental chromium in stress situations such as those examined in these trials is more thoroughly addressed in a review by Burton [81].

Chang et al. supplemented diets for older calves with CrYst for 138 days at the rate of 0 or 200 ppb Cr and observed no effects of supplementation on growth performance or carcass traits [82]. This is a relatively stress-free period of growth. Young dairy steers were used by Besong et al. with supplementation of 800 ppb Cr from CrPic [83]. The authors observed some differences in serum constituents throughout the 9-week study (though there was no pattern to the values over time) and lower triglycerides levels in the liver at week 9, but there were no effects on growth performance.

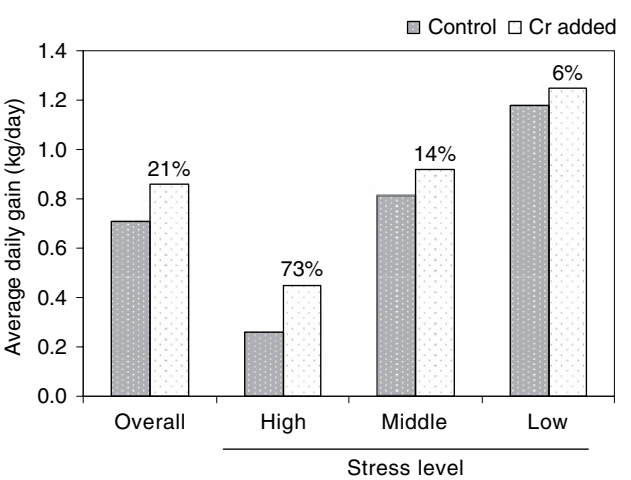



Fig. 1. Effect of Cr supplementation on growth rate of calves during the first 28–30 days in the feedlot (adapted from Mowat, 1997) [80].

### Chromium for dairy cows

Dairy cows have demonstrated positive immune responses in late gestation and early lactation to the supplementation of Cr from an amino acid chelate [84], and first parity cows have responded to supplemental amino acid chelated Cr with a 13% increase in milk yield during the first 16 weeks of lactation [85]. Hayirli et al. examined the pre- and post-parturient responses to supplemental Cr from CrMeth supplemented at 0, 0.03, 0.06, and 0.12 mg/kg metabolic BW and observed different results for the two periods [86]. CrMeth supplementation improved glucose tolerance postpartum but not prepartum. Basal insulin levels were actually elevated by Cr supplementation ($P < 0.06$) prepartum and plasma NEFA levels were decreased linearly ($P < 0.03$). This contrasts to work where Cr from CrPic was supplemented at about 2000 ppb to dairy cows [87]. These researchers did not observe any generalized change in glucose or insulin measures, but they did observe an increase in glucose clearance rate ($P < 0.06$) and a trend toward decreased half-life ($P < 0.10$) in primiparous cows that was not present in multiparous cows (this effect in young cows but absence in older cows is consistent with Yang et al. [85]).

An occasional problem that occurs at calving in dairy cows is a prolonged expulsion of fetal membranes. This condition is called "retained placenta" and can be associated with a variety of metabolic and rebreeding problems; one of the associated hormonal changes that occurs concomitant with this condition is an increase in cortisol concentrations. This led Villabos et al. to examine the effects of supplementation of 3.5 mg Cr/day from CrPic to cows for 9 weeks prior to expected calving date in a herd that had historical problems with the malady. The supplementation reduced the observed incidence of retained placenta from 56% in unsupplemented cows to 16% in supplemented cows ($P < 0.01$) [88].

### Summary for cattle

Chromium supplementation of cattle has resulted in some dissimilarities as well as similarities of response to that of pigs. Interestingly, occasions of insulin increase have been reported when supplementing Cr to these ruminants. Supplementation has not demonstrated improvements in growth measures unless growth is depressed for some reason. Supplementation appears to be of primary benefit in stressful situations. Because the nature of these stresses varies (e.g., antigen exposure, psychological stress of regrouping animals, metabolic stresses of the initiation of lactation), the physiologic and endocrinologic changes in the body would be many. Which of these changes, or whether all of the changes, are potentially impacted by Cr supplementation is not clear; however, situations in which the stress is associated with cortisol may be situations of potential response to supplementation.

## POULTRY

### Introduction

The poultry diet will be similar to the diet of pigs. It is a grain-based diet that will yield primarily glucose upon digestion and absorption. The avian species is very different, however, from mammals with regard to basal glucose levels, ratio of insulin to glucagon cells in the pancreas, and control of insulin release [89, 90, 91]. An understanding

of glucose and insulin as it relates to metabolism and growth is less complete than in mammals. Basal glucose levels in chickens of 180–230 mg/dL would be relatively normal [92, 93], and levels of several hundred could be found in smaller avians.

Chickens that are raised for meat production are called broilers. They will be about 40 grams at hatching and will be marketed at a weight of 2.4 kg at about 6 weeks of age in the United States. During this period of growth, mortality will be about 4–5% of the flock depending on a variety of factors that may be related to diet, crowding, disease pressure, environmental stress (heat or cold), etc. Chickens that are raised for egg production are called laying hens or layers. They will begin laying eggs at about 19–20 weeks of age and will generally lay for a minimum of 13–14 months. The average laying percentage (number of eggs per day/number of layers in the group) will start out low and increase to 93–94%, averaging 82–84% over the productive life of the hen.

**Chromium for broilers (meat birds)**

Early studies with supplemental inorganic $Cr^{+3}$ ($CrCl_3 \cdot 6H_2O$) at levels of 20 ppm to young turkey poults demonstrated some improvement in growth rate and feed efficiency [94, 95]. Ward et al. reported a tendency for 200 ppb organic Cr (from CrPic) to increase carcass lean percentage and decrease fat percentage in young 3-week-old broiler chicks [96]. A study from Brazil where CrYst was supplemented from day 28 post-hatching through slaughter at day 47 did demonstrate ($P < 0.05$) a reduction in fat percentage of breast meat when the birds were supplemented with 400 ppb Cr from CrYst [97]; additionally, a 6-point improvement in feed efficiency (2.14 vs 2.20; $P < 0.05$) was observed with the supplementation. While this effect is encouraging, another study in the same paper reported no effect of 300 or 600 ppb from CrYst on growth or feed efficiency when it was supplemented only during the first 21 days of life. As in the discussion of the effects in pigs, it should be noted that effects on carcass composition are cumulative effects; therefore, the length of feeding and relative age at slaughter are important factors relative to conclusions about the ability of a Cr form to effect carcass responses. A third study that evaluated feeding during both the first and the second 3 weeks showed minor improvements in both the periods. The last study also demonstrated reductions in mortality in the groups supplemented with Cr. Both the reduction in mortality and the feed efficiency response have potentially large impacts on profitability. Studies from the Czech Republic have reported improvements in growth rate and feed efficiency of turkeys supplemented with 400 or 2000 ppb of CrPic or chromium nicotinate for 88 days [98]. There were no differences between level or form of Cr. All supplemented birds had lower fat content in breast meat.

Lien et al. utilized broilers to evaluate the effects of 0, 800, 1600, and 3200 ppb Cr from CrPic on growth and carcass responses [99]. Supplementation of 800 ppb had little effect, but 1600 ppb Cr increased growth rate with 3200 ppb supplementation having an intermediate effect. Both of the two upper levels did not affect feed efficiency as growth effects were primarily through increases in voluntary feed intake. The two highest levels of Cr increased liver fat percentage and decreased abdominal fat percentage. With regard to serum lipid responses, increasing levels of Cr resulted in numerical reductions in serum triglycerides and cholesterol and significant reductions in NEFA; a greater percentage of the lipoproteins existed as HDL (compared to LDL and VLDL) with increasing levels of Cr, but there was no difference in the proportion of total cholesterol bound to HDL.

A dose–response study was also conducted by Lee et al. who evaluated 0, 200, 400, and 800 ppb Cr from CrPic on growth performance, bone traits, serum traits, and immune responses of broilers [100]. Two trials were conducted. In the first trial the Cr was supplemented for 6 weeks while in the second trial it was supplemented for only 2 weeks. Neither trial exhibited effects of supplementation on growth traits but in both trials there was a numerical increase in total cholesterol at the end of the supplementation period and a significant ($P < 0.07$) increase in HDL-cholesterol (the "good" cholesterol). With regard to immune response, no differences were observed at 3 weeks of age (birds received antigen exposure at 4 and 14 days); but at 6 weeks of age, higher titers were observed in birds in which supplementation was continued (Trial 1; $P < 0.06$) but not in birds in which supplementation had been discontinued (Trial 2). Supplementing 400 and 600 ppb Cr from CrYst, Wang et al. also observed no effect on growth or carcass traits but did observe decreased serum TG, total cholesterol, HDL-cholesterol, and FFA at both 3 and 7 weeks of measurement with the effects more pronounced with longer feeding [101]. In support, Kim et al. reported two studies in which graded levels of Cr picolinate were fed to broilers for a 6-week period with the result that total serum cholesterol was decreased quadratically and HDL cholesterol increased quadratically [102, 103]; neither growth performance nor efficiency of nutrient utilization was improved, but mortality was reduced in both studies with the addition of the Cr. Yeswanth evaluated incremental additions of CrYst up to 300 ppb and observed a linear decrease in abdominal fat percentage and a linear increase in HDL cholesterol [104].

So results with broilers do not always demonstrate an effect on performance – additional research is needed to determine the situations most responsive to the supplementation of the Cr, but there does seem to be an effect on serum lipids relatively often.

Not all studies report the mortality that occurs during the study; however, in those studies that have reported it, there seems to be a relatively uniform effect in reducing mortality that, obviously, has major effects on profitability. Table 4 presents those trials in which mortality is reported. The first two trials are from Brazil where the baseline mortality is low but there are still small improvements with supplementation of the broiler diets. The third and fourth trials are from Korea using CrPic supplementation of broiler diets. Baseline mortality is higher, and the response to supplementation is larger; it is interesting that the title of the manuscript for Trial 3 has the term "excessive chromium" which suggests the authors may have postulated potential adverse effects

Table 4
Effect of Cr supplementation on poultry mortality

| Cr level, ppb: | 0 | 100 | 150 | 200 | 300 | 400 | 600 | 800 | 1600 | 2400 |
|---|---|---|---|---|---|---|---|---|---|---|
| Trial 1 – CrYst | 1.04 | | | | | 0.78 | | | | |
| Trial 2 – CrYst | 3.85 | | 3.60 | | 2.00 | | | | | |
| Trial 3 – CrPic* | 8.3 | | | | | | | 5.5 | 0 | 0 |
| Trial 4 – CrPic* | 12.5 | 6.3 | | 4.2 | | 4.2 | 2.1 | 0 | | |
| Trial 5 – CrPic* | 6.25 | | | 5.0 | | 5.42 | | 2.92 | | |

Trial 1, 2 – Hossain et al. [97]; Trial 3, 4 – Kim et al. [102, 103]; Trial 5 – Kim et al. [105].
* Cr effect ($P < 0.05$).

rather than the positive effects that were observed; indeed, it is interesting that higher levels of Cr actually eliminated mortality in two of the studies. While it cannot be said that Cr supplementation would consistently eliminate mortality (because the totality of the physiologic reasons for the mortality are not characterized and, even if characterized, could not be assumed to be the reasons for mortality in other situations), it certainly can be said that levels of supplementation in these studies were not "excessive" levels that should cause concern about overall well-being. The final trial is with older birds (laying hens) and also demonstrates improvements. Several points can be made from the trials. First, when mortality is low (and presumably the cumulative stress from crowding, disease challenge, heat, etc. is low), there is less of a response to supplemental Cr; but when mortality (and presumably stress load) is high, then the response to Cr can be quite large. A second point to be made is that the response is clearly dose dependent.

The effect of a disease stress on the response to Cr has actually been evaluated [106]. The researchers used a turkey poult model in which young poults were infected with a diarrheal disease that causes enteritis resulting in poor performance and increased mortality. The addition of 400 ppb Cr from CrYst did not reduce mortality significantly (although there was a numeric reduction), but it restored about 25% of the reduced growth rate and 66% of the feed efficiency that was reduced with the disease. Additionally, the disease reduced plasma levels of $T_3$, $T_4$, IGF-I, and IGF-II, all of which were restored partially ($T_4$) or completely with the supplementation of chromium. In another stress model that utilized ambient temperature extremes outside of the zone of thermalneutrality for broiler chicks (note: this temperature stress will reduce thyroid activity), Sahin et al. also demonstrated increased serum $T_3$ and $T_4$ in response to 400 ppb Cr from CrPic [107]; this change in thyroid hormone level was associated with improvements in feed intake and weight gain. The relationship between Cr, thyroid response, feed intake, and weight gain appears to be linear up to 1200 ppb Cr [108]. Sands and Smith also report a partial restoration of growth with 200 and 400 ppb supplemental Cr from CrPic in situations of heat stress [109].

**Chromium for laying hens**

An exciting piece of research involved supplementation of year-old layers with 0, 200, 400, or 800 ppb Cr from CrPic (Table 5) [110]. Supplementation resulted in lower total serum cholesterol in the layers and, also, resulted in a reduction in egg yolk cholesterol. However, eggshell-breaking strength was reduced with Cr supplementation (suggesting interrelationships with aspects of metabolism of other minerals) that certainly should require further examination. Lien et al. continued examination of the effects of Cr supplementation on lipids in laying hens [111, 112]. In the first study [111], the supplementation of 1000 ppb Cr from CrPic to 45-week-old hens resulted in numeric decreases in triglycerides and cholesterol in hen serum and significant decreases in yolk cholesterol; Cr supplementation also increased the proportion of lipoproteins as HDL and increased the percentage of cholesterol bound as HDL-cholesterol. The second study [112] utilized multiple levels of CrPic (0, 800, and 1600 ppb) with 45-week-old layers and observed no effect on egg production or egg quality (egg weight, eggshell strength, or thickness) but a decrease in egg yolk cholesterol ($P < 0.06$) with Cr supplementation. And, as in the previous study, there were numeric decreases in serum triglycerides and cholesterol of the hens that were associated with significant reductions in VLDL

Table 5
Effect of Cr from CrPic on egg production and serum traits in laying hens

| | Chromium (ppb) | | | |
|---|---|---|---|---|
| | 0 | 200 | 400 | 800 |
| Egg production (%) | 81.2 | 79.4 | 81.4 | 79.0 |
| Egg weight (g) | 68.5[a] | 60.6[b] | 66.0[a] | 64.5[a,b] |
| Eggshell breaking strength (kg/cm) | 2.02[a] | 1.77[a,b] | 1.34[b] | 1.42[b] |
| Yolk cholesterol (mg/g) | 23.1[a] | 19.9[a] | 16.5[a,b] | 15.3[b] |
| Total serum cholesterol (mg/dl) | 123[a] | 125[a] | 114[a] | 75[b] |
| HDL-cholesterol (mg/dl) | 13[a] | 17[a] | 17[a] | 22[b] |
| LDL-cholesterol (mg/dl) | 81[a] | 61[b] | 30[c] | 25[c] |

Adapted from Lien et al. [110]
[a,b] Means within a row without a common superscript differ ($P < 0.05$).

percentage and increases in HDL percentage. However, unlike the previous study, there was a difference in which lipoprotein subgroup carried the cholesterol in that there was an increase in the percentage of the total cholesterol bound to the VLDL. The authors did note that they used a different method for quantifying the VLDL-C than was used in the first study, but it is unlikely that laboratory methodology would explain these differences in treatment effects related to the lipoprotein fraction to which the cholesterol was bound.

Another interesting study is reported from China [113] where researchers also note effects on serum and egg yolk cholesterol in broiler breeder hens (hens which lay fertile eggs that are incubated and hatched for broiler chicks which are raised for meat production) rather than laying hens (which lay unfertile eggs that are sold for human consumption). In addition, positive effects on egg hatchability with supplementation of Cr yeast were observed (Table 6). The interesting point about the hatchability improvement is that control birds initially have low hatchability that increases over about a 2-week period; however, Cr-supplemented layers had peak hatchability at the beginning of the observational period. This suggests that some of the initial low performance, as the bird adapts from a non-laying stage of life to the new physiological situation of laying eggs, is related to a less-than-optimal Cr status.

Kim et al. evaluated the effects of CrPic in a study with 960 laying hens. Supplementation levels of 0, 200, 400, and 800 ppb Cr were evaluated in conjunction with dietary protein level [105]. Effects on egg production and egg characteristics were relatively non-descript; however, there were improvements in utilization of several nutrients with Cr supplementation (Table 7) and were reductions in mortality in supplemented groups (noted on Table 4). An improvement in feed efficiency (which would presumably be accomplished through improved nutrient digestibility) with no net increase in egg production has also been observed in India [114] by Mathivanan and Selvaraj when supplementing 0, 250, 500, and 750 ppb Cr from CrPic. Improved dry matter, protein, and fat digestibility has also been observed with the addition of 400 ppb Cr from CrPic [115, 116] and with 400 and 800 ppb from CrPic [117].

Table 6
Effects of supplementation of CrYst to layers

| | Chromium from CrYst (ppb) | | |
|---|---|---|---|
| | 0 | 400 | 800 |
| Study 1 | | | |
| Egg production (%) | 83.7 | 82.5 | 82.5 |
| Serum | | | |
| Total cholesterol (mg/dl) | 109.7 | 68.9 | 66.3 |
| HDL-cholesterol (mg/dl) | 16.0 | 61.1 | 62.4 |
| Yolk cholesterol (mg/100 g) (d 30) | 1673 | 1356 | 1271 |
| Study 2 | | | |
| Egg hatchability (%) | | | |
| day 15–18 | 78.3 | 91.7 | 90.0 |
| day 19–22 | 86.8 | 96.7 | 94.3 |
| day 23–26 | 92.9 | 90.9 | 96.4 |
| Egg cholesterol (mg/100 g) (d 30) | 1521 | 1138 | 1086 |

Adapted from Xianlin and Fanping [113].

Table 7
Effects of dietary CrPic on the nutrient utilization of brown laying hens (37–43 weeks)

| Items | Chromium from CrPic (ppb) | | | |
|---|---|---|---|---|
| | 0 | 200 | 400 | 800 |
| Energy | 87.83 | 87.46 | 88.50 | 88.39 |
| Dry matter | 82.62[a] | 83.75[a,b] | 85.27[b] | 84.65[b] |
| Crude protein | 80.02[a] | 83.43[b] | 84.48[b] | 84.75[b] |
| Crude fat | 92.21 | 91.51 | 91.27 | 91.47 |
| Crude ash | 60.91 | 60.83 | 63.80 | 61.46 |

Adapted from Kim et al. [105].
[a,b] Values without a common superscript within the same row differ, $P < 0.05$.

## Summary

Cr supplementation of poultry has generated some of the same type of results as in pigs. Supplementation of the very young has limited effect, but the older growing bird occasionally shows improvements in carcass measures. Like cattle, situations of stress appear to be responsive to Cr supplementation in a dose-related manner with very clear improvements in overall well-being as indicated by marked reductions in mortality in both broiler and laying-hen studies. The work with poultry has demonstrated relatively consistent effects in serum lipid profiles and, occasionally, in cholesterol-related measures.

## SHEEP

### Introduction

Sheep do not represent a major livestock species at the present time in North America though they are important in other parts of the world and did constitute a larger part of the meat consumption in this part of the world in the late 1800s and early 1900s. Sheep are grazing animals, consume a diet similar to that of cattle, and have an anatomy similar to cattle. Thus serum glucose levels are, like cattle, only about half that of pigs and people (44–81 mg/dL) [72].

### Metabolic effects

Kitchalong et al. evaluated the response of growing lambs to supplementation of 250 ppb Cr from CrPic with the classic IVGTT and IVICT [118]. They did not observe any differences in glucose clearance rate or half-life during these tests; however, plasma insulin was elevated ($P < 0.05$) and plasma glucose was reduced ($P < 0.07$) during the IVGTT at week 2. Over the course of the 11-week period of feeding, NEFA were consistently lower in Cr-fed lambs. As in some of the growing poultry and pig research, there was no effect of dietary supplementation on growth rate but there was a slight reduction in carcass fat measures. Forbes et al. examined the potential for there to be a different response to 370 ppb Cr from CrPic based on the breed of sheep (meat breed vs wool breed) and failed to find a difference [119]. They observed breed differences but not in response to the supplementation of the Cr. The Cr did not affect growth performance or any aspect of glucose kinetics in response to an IVGTT; Cr supplementation did decrease fasting plasma NEFA ($P < 0.04$) prior to the IVGTT.

### Chromium for growing lambs

Olsen et al. also observed no growth effects related to the supplementation of 500 or 1000 ppb Cr from CrPic in sheep as well as no improvements in muscling or fat measures [120]. In further research with the supplementation of 400 ppb Cr from CrPic to castrate lambs at two levels of protein in the diet [121], there were inconsistent effects on both production and metabolic responses measured.

### Summary

Of all the farm livestock evaluated, sheep have demonstrated less potential for supplementation than any other species. As with the other ruminant species reviewed, cattle, Cr supplementation has been noted to increase insulin at times.

## EQUINE

### Introduction

Very little research has been reported with horses compared to other species. Horses are different than other farm livestock in that they primarily consume forage (like cattle and sheep) but ferment the forage in the cecum and large intestine rather than in an enlarged, multi-compartmented stomach prior to the small intestine (as cattle and sheep do). Thus, the anatomy of the horse is closer to the pig, but its diet is closer to a cow. Serum glucose levels are intermediate (62–114 mg/dL) [72] to that of the ruminants (i.e., the cattle and sheep) and that of pigs and people.

**Metabolic effects**

Even with these differences, horses have been demonstrated to have a dose-dependent increase in glucose fractional turnover rate and reduction in glucose half-life following an IVGTT when diets were supplemented at the rate of 105, 210, and 420 ppb Cr from CrPic [122]. Socha et al. used a different manner of supplementing horses, choosing to supply 0, 0.005, 0.01, and 0.02 mg Cr/kg BW with CrMeth, but they also observed improvements in certain indices of glucose metabolism [123]. Additionally, they noted that glucose responses to a normal meal may be a more sensitive indicator of glucose/insulin tolerance than responses to an IVGTT.

**Chromium for horses**

A report by Pagan et al. of a positive response to CrYst supplementation in exercising thoroughbred horses is exciting and the responses are consistent with those observed in other species [124]. Using a switchback design in which the horses received 5 mg/day of Cr from CrYst when they were being fed the supplemented diet, benefits were observed in several metabolic responses during periods of exercise on a treadmill that were not always evident during non-exercise periods (Fig. 2). During the exercise period, the supplementation of Cr resulted in observations of a reduction of plasma glucose ($P < 0.05$), a reduction of plasma lactate ($P < 0.10$), a reduction of plasma cortisol ($P < 0.05$), and an elevation of plasma triglycerides ($P < 0.10$). While the observations

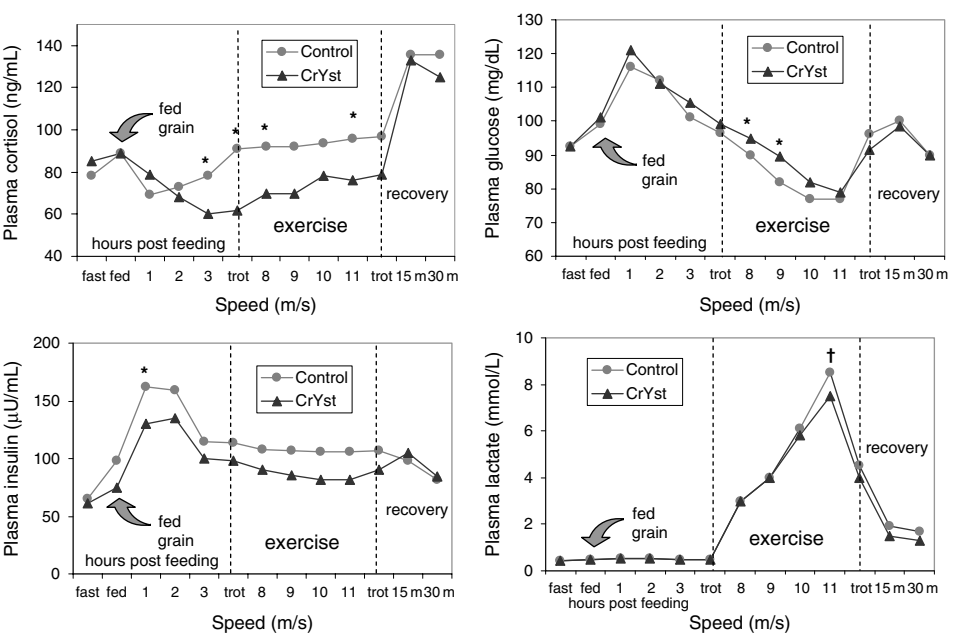



Fig. 2. Effect of Cr from CrYst on serum responses of thoroughbreds during treadmill; significant effects at individual time points are denoted by an * ($P < 0.05$) or by an † ($P < 0.10$) (adapted from Pagan et al. [124]).

were not statistically significant at each time point measured, the shape of the curve was very consistent and demonstrated more efficient energy utilization due to the supplementation of Cr. The effects on plasma insulin levels demonstrate a consistent effect on the values over time. This type of response, in a situation in which a type of stress exists, is consistent with results in other species wherein the response to Cr is sometimes small or unobservable in a non-stress situation but is magnified under stress.

## SUMMARY

### Qualitative responses

Farm livestock respond to Cr supplementation in a variety of ways. All species evaluated have demonstrated some manner of response to classic IVGTT or IVICT but the responses differ by species and by magnitude. In almost all species, there is little effect on growth rate unless that growth rate has been severely compromised by some type of stressor.

Beyond the effects on glucose and insulin, Cr supplementation has clearly affected serum cortisol levels in multiple species. Whether this is a direct effect of Cr on the adrenal gland or an indirect effect via alterations of insulin is not determined. However, the adverse effects of excess glucocorticoids such as cortisol are well known. The excesses inhibit fibroblasts which can lead to a loss of collagen and connective tissue, which then can manifest itself as easy bruising and poor wound healing. Negative effects can also be observed on bone formation and calcium absorption, as well as general growth and development in children. A host of other effects related to immunocompetence, renal function, cardiovascular function, and decreased libido are known. But, while these effects of gross glucocorticoid excess are known, whether the smaller moderations that result from Cr supplementation will have effects on long-term health and well-being are less certain.

The interrelationship of Cr with serum lipids and cholesterol should not be overlooked. The effects in farm livestock have primarily been with avians; but because cholesterol is a concern among the human population because of its relationship with, and implications in, cardiovascular disease, it should receive greater attention as a research subject.

Improvements in reproducing animals of many species have been noted. Improvements have been observed in fecundity (pigs), in situations related to health issues at birth (retained placenta in dairy cows) and fertility (hatchability of eggs). Reproduction may be a fruitful area of continued research, especially in situations of reduced capacity or subnormal reproductive performance.

The improvements in situations of known stress ("shipping stress" in young feedlot calves and that which results in broiler mortality) are some of the most exciting and represent the pooled benefits of Cr on animal/avian well-being. Apportioning those benefits into those related to glucose/insulin metabolism, steroid metabolism, immunocompetence, and so on is difficult but provides hope for many areas of research.

### Quantitative responses

The fact that all of the subjects in some studies were not aided by the Cr supplementation demonstrates that Cr is not a drug which would have the general effect

of altering some parameter in all subjects; Cr is a nutrient which, when supplemented, benefits animals that are deficient while providing little or no benefit to those that are not deficient. While this means that situations of what the stockman considers "normal" may not all respond to supplementation, it should still be noted that the research community does not totally understand what is indeed "normal good health or performance" versus what is simply familiarity with performance or health in Cr-deficient livestock. The research does illustrate that a good portion of subjects with low performance or suffering from some adverse health effects do so because of inadequate Cr nutrition. To the extent, then, that these situations result from the Cr status of the subject, they can be alleviated with proper Cr supplementation.

Time/dose relationships are evident in several of the results in multiple species (litter size in pigs, mortality in broilers, cortisol in stressed calves, lipid responses in poultry). Cr supply relative to body mass may explain some of the inconsistencies in response to this point and may be a fruitful area of research.

**General conclusion**

The results from multiple species demonstrate broad effects of Cr on the physiological status of animals. While patterns of responses may be evident and there may be consistencies across species, responses are not guaranteed by supplementation; individual responses appear to be governed by several factors. Although there are currently only four forms of Cr that can be marketed in North America and only two species supplemented, this will undoubtedly increase in the future as other forms of Cr are determined to be biologically available and as continuing research establishes species or situations that are responsive to supplementation. Additionally, the allowed limits of supplementation may change based upon research that identifies production situations in which the apparent need of the animal for the mineral Cr is elevated. After one decade of supplementation, the future remains bright.

## ACKNOWLEDGMENTS

Work on Cr(III) under the direction of M. D. L. has been funded by the Virginia Pork Industry Board, the National Pork Producer's Council, and Prince AgriProducts, Inc.

## REFERENCES

[1] Anderson, R. A. (1987) Chromium in *Trace Elements in Human and Animal Nutrition* (Mertz, W. Ed.) Vol. 1, pp. 225–244. Academic Press, Inc., San Diego, CA.

[2] National Research Council (NRC) (1989) *Recommended Dietary Allowances* (10th edn) National Academy of Sciences, National Academy Press, Washington, D.C.

[3] Anderson, R. A. (1994) Stress effects on chromium nutrition of humans and farm animals in *Proc. Alltech's Tenth Ann. Symp.* (Lyons, T. P., and Jacques, K. A., Eds) pp. 267—274. Nottingham University Press, Loughborough.

[4] Mahalko, J. R., and Bennion, M. (1976) The effect of parity and time between pregnancies on maternal hair chromium concentration. *Am. J. Clin. Nutr. 29*, 1069–1072.

[5] Mertz, W., and Roginski, E. E. (1969) Effects of chromium(III) supplementation on growth and survival under stress in rats fed low protein diets. *J. Nutr. 97*, 531–536.

[6] National Research Council (NRC) (1997) *The Role of Chromium in Animal Nutrition*. National Academy of Sciences, National Academy Press, Washington, D.C.
[7] Pond, W. G., and Lei, X. G. (2000) Of pigs and people in *Swine Nutrition* (Lewis, A. J., and Southern, L. L., Eds) pp. 3–18. CRC Press, Boca Raton, FL.
[8] Phillips, R. W., and Panepinto, L. M. (1986) Swine as a model for human diabetes in *Swine in Biomedical Research* (Tumbleson, M. E., Ed.) Vol. 1, pp. 549–560. Plenum Press, New York.
[9] Pond, W. G., and Houpt, K. A. (1978) *The Biology of the Pig*. Cornell University Press, Cornell, NY.
[10] Bunding, I. M., Davenport, M. E., Jr., and Schooley, M. A. (1956) The glucose tolerance test in swine and its implications. *J. Anim. Sci. 15*, 234–241.
[11] Machlin, L. J., Horino, M., Hertelendy, F., and Kipnis, D. M. (1968) Plasma growth hormone and insulin levels in the pig. *Endocrinol. 82*, 369–376.
[12] Amoikon, E. K., Fernandez, J. M., Southern, L. L., Thompson, D. L., Jr., Ward, T. L., and Olcott, B. M. (1995) Effect of chromium tripicolinate on growth, glucose tolerance, insulin sensitivity, plasma metabolites, and growth hormone in pigs. *J. Anim. Sci. 73*, 1123–1130.
[13] Lindemann, M. D., Wood, C. M., Harper, A. F., Kornegay, E. T., and Anderson, R. A. (1995) Dietary chromium picolinate additions improve gain: Feed and carcass characteristics in growing-finishing pigs and increase litter size in reproducing sows. *J. Anim. Sci. 73*, 457–465.
[14] Matthews, J. O., Southern, L. L., Fernandez, J. M., Pontif, J. E., Bidner, T. D., and Odgaard, R. L. (2001) Effect of chromium picolinate and chromium propionate on glucose and insulin kinetics of growing barrows and on growth and carcass traits of growing-finishing barrows. *J. Anim. Sci. 79*, 2172–2178.
[15] Guan, X., Matte, J. J., Ku, P. K., Snow, J. L., Burton, J. L., and Trottier, N. L. (2000) High chromium yeast supplementation improves glucose tolerance in pigs by decreasing hepatic extraction of insulin. *J. Nutr. 130*, 1274–1279.
[16] Fakler, T. M., Ward, T. L., Kegley, E. B., Socha, M. T., Johnson, A. B., and Maxwell, C. V. (1999) Metabolic effects of dietary chromium-L-methionine in growing pigs in *Proc. 10th Intl. Symp. On Trace Elements in Man and Animal*, *May 2–7*, p. 110. Evian, France.
[17] Evock-Clover, C. M., Polansky, M. M., Anderson, R. A., and Steele, N. C. (1993) Dietary chromium supplementation with or without somatotropin treatment alters serum hormones and metabolites in growing pigs without affecting growth performance. *J. Nutr. 123*, 1504–1512.
[18] Garcia, M. R., Newcomb, M. D., and Trout, W. E. (1997) Effects of dietary chromium picolinate supplementation on glucose tolerance and ovarian and uterine function in gilts. *J. Anim. Sci. 75*, 82.
[19] Lee, D. N., Yen, H. T., Shen, T. F., and Chen, B. J. (1997) The effects of chromium picolinate supplementation on growth performance and immunity response of weanling pigs. *J. Chin. Soc. Anim. Sci. 26*, 373–386.
[20] Lee, D. N., Yen, H. T., Shen, T. F., and Chen, B. J. (1999) The effect of chromium picolinate supplementation on the lymphocyte subsets of weanling pigs. *J. Agric. Assoc. China 187*, 1–9.
[21] Lee, D. N., Chen, B. J., Yen, H. T., and Shen, T. F. (1999) Effects of chromium picolinate supplementation on interleukin-2 activity of peripheral blood mononuclear cells in vitro and the physiological response to lipopolysaccharide injection in weanling pigs. *J. Chin. Soc. Anim. Sci. 28*, 277–288.
[22] Lee, D. N., Lin, Y. A., Huang, S. C., Lin, Y. P., and Weng, C. F. (2000) Effects of dietary chromium picolinate supplementation on growth performance, plasma constituents, and disease resistance in weanling pigs. *J. Agric. Assoc. China 1*, 42–53.

[23] van Heugten, E. V., and Spears, J. W. (1997) Immune response and growth of stressed weanling pigs fed diets supplemented with organic or inorganic forms of chromium. *J. Anim. Sci. 75*, 409–416.

[24] Tang, L., Li, D., Wang, F. L., Xing, J. J., and Gong, L. M. (2001) Effects of different sources of organic chromium on immune function in weaned pigs. *Asian-Aust. J. Anim. Sci. 14*, 1164–1169.

[25] Lee, D. N., Shen, T. F., Yen, H. T., Weng, C. F., and Chen, B. J. (2000) Effects of chromium supplementation and lipopolysaccharide injection on the immune responses of weanling pigs. *Asian-Aust. J. Anim. Sci. 13*, 1414–1421.

[26] Lee, D. N., Weng, C. F., Yen, H. T., Shen, T. F., and Chen, B. J. (2000) Effects of chromium supplementation and lipopolysaccharide injection on physiological responses of weanling pigs. *Asian-Aust. J. Anim. Sci. 13*, 528–534.

[27] van de Ligt, J. L., Lindemann, M. D., Harmon, R. J., Monegue, H. J., and Cromwell, G. L. (2002) Effect of chromium tripicolinate supplementation on porcine immune response during the periparturient and neonatal period. *J. Anim. Sci. 80*, 456–466.

[28] van de Ligt, J. L., Lindemann, M. D., Harmon, R. J., Monegue, H. J., and Cromwell, G. L. (2002) Effect of chromium tripicolinate supplementation on porcine immune response during the postweaning period. *J. Anim. Sci. 80*, 449–455.

[29] Cho, W. T., Han, I. K., Chae, B. J., Han, Y. K., Ha, J. K., and Odle, J. (2000) Effects of chromium picolinate, L-carnitine and thyroxine on the performance, nutrient digestibility and nitrogen balance in pigs weaned at 21 days of age. *J. Anim. Feed Sci. 9*, 633–645.

[30] Cho, W. T., Kim, J. H., Kang, W. B., Han, I. K., and Han, Y. K. (1999) Effects of L-carnitine, chromium picolinate with different fat sources on growth and nutrient digestibility in pigs weaned at 21 days of age. *Kor. J. Anim. Sci. 41*, 445–456.

[31] Paul, R., Garner, J. M., and Sweet, O. K. (1991) *The Pork Story*. National Pork Producers Council, p. 30. Lowell Press, Inc., Kansas City, MO.

[32] Page, T. G., Southern, L. L., Ward, T. L., and Thompson, D. L., Jr. (1993) Effect of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. *J. Anim. Sci. 71*, 656–662.

[33] Page, T. G., Ward, T. L., and Southern, L. L. (1990) Chromium supplementation of corn-soybean meal diets for finishing swine. *J. Anim. Sci. 68 (Suppl. 1)*, 218.

[34] Lindemann, M. D. 1999. Chromium and swine nutrition. *J. Trace Elem. Exp. Med. 12*, 149–161.

[35] National Research Council (NRC) (1988) *Nutrient Requirements of Swine* (9th edn). National Academy Press, Washington, D.C.

[36] Kornegay, E. T., Wang, Z., Wood, C. M., and Lindemann, M. D. (1997) Supplemental chromium picolinate influences nitrogen balance, dry matter digestibility, and carcass traits in growing-finishing pigs. *J. Anim. Sci. 75*, 1319–1323.

[37] Liarn, T., Chen, S., Chen, C., and Wu, C. 1993. The Effects of various levels of chromium picolinate on growth performances and serum traits of pigs. *J. Chin. Soc. Anim. Sci. 22*, 349–357.

[38] Lien, T., Wu, C., Lu, J., and Chou, R. (1996) Effects of supplemental chromium picollnate on growth performances, serum traits and carcass characteristics of pigs. *J. Chin. Soc. Anim. Sci. 25*, 253–260.

[39] Min, J. K., Kim, W. Y., Chae, B. J., Chung, I. B., Shin, I. S., Choi, Y. J., and Han, I. K. (1997) Effects of chromium picolinate (CrPic) on growth performance, carcass characteristics and serum traits in growing-finishing pigs. *Asian-Aust. J. Anim. Sci. 10*, 8–14.

[40] Ward, T. L., Southern, L. L., and Bidner, T. D. (1997) Interactive effects of dietary chromium tripicolinate and crude protein level in growing-finishing pigs provided inadequate and adequate pen space. *J. Anim. Sci. 75*, 1001–1008.

[41] Gundel, J., Herman, I., Regiusne, M. A., Votisky, L., Vigh, L., and Szelenyine, G. M. (1998) Effect of chromium picolinate feeding production traits on growing-fattening pig. *Allattenyesztes es Takarmanyozas 47*, 337–350.

[42] Lien, T., Wu, C., Lin, B., Wang, B., Lu, J., and Shiao, T. (1998) Effect of different protein and limiting amino acid levels coupled with a supplement of chromium picolinate on lipid metabolism and carcass characteristics of pigs. *J. Anim. Sci. 67*, 601–607.

[43] Hanczakowska, E., Urbańczyk, J., and Światkiewicz, M. (1999) The efficiency of betaine and organic compounds of chromium in fattening of pigs with ad libitum or restricted feeding. *Ann. Anim. Sci. – Rocz. Nauk. Zoot. 26*, 263–274.

[44] Lien, T. F., Wu, C. P., Wang, B. J., Shiao, M. S., Shiao, T. Y., Lin, B. H., Lu, J. J., and Hu, C. Y. (2001) Effect of supplemental levels of chromium picolinate on the growth performance, serum traits, carcass characteristics and lipid metabolism of growing-finishing pigs. *J. Anim. Sci. 72*, 289–296.

[45] Urbańczyk, J., Hanczakowska, E., and Światkiewicz, M. (2001) Effect of energy concentration on the efficiency of betaine and chromium picolinate as dietary supplements for fattening pigs. *J. Anim. Feed Sci. 10*, 471–484.

[46] Matthews, J. O., Higbie, A. D., Southern, L. L., Coombs, D. F., Bidner, T. D., and Odgaard, R. L. (2003) Effect of chromium propionate and metabolizable energy on growth, carcass traits, and pork quality of growing-finishing pigs. *J. Anim. Sci. 81*, 191–196.

[47] Shelton, J. L., Payne, R. L., Johnston, S. L., Bidner, T. D., Southern, L. L., Odgaard, R. L., and Page, T. G. (2003) Effect of chromium propionate on growth, carcass traits, pork quality, and plasma metabolites in growing-finishing pigs. *J. Anim. Sci. 81*, 2515–2524.

[48] Matthews, J. O., Guzik, A. C., LeMieux, F. M., Southern, L. L., and Bidner, T. D. (2005) Effects of chromium propionate on growth, carcass traits, and pork quality of growing-finishing pigs. *J. Anim. Sci. 83*, 858–862.

[49] Simek, M., Zemanova, D., and Dvorak, J. (1996) Effect of chromium supplementation on pig performance and carcass quality in *Proceedings of the European Association of Animal Producers Meetings*, Norway.

[50] Lemme, A., Wenk, C., Lindemann, M. D., and Bee, G. (1999) Chromium yeast affects growth performance but not whole carcass composition of growing-finishing pigs. *Annales de Zootechnie 48*, 457–468.

[51] Lemme, A., Wenk, C., Lindemann, M., and Bee, G. (2000) Chromium yeast affects growth performance and plasma traits but not carcass characteristics of growing-finishing pigs depending on the glycemic index. *Arch. Tierernahr. 53*, 157–177.

[52] Renteria, F. J. A., and Cuaron, I. J. A. (1998) Chromium picolinate in the diet of growing-finishing swine. *Tech. Pecu. Mex. 36*, 121–139.

[53] George, P. B., England, D. C., Siers, D. G., and Stanton, H. C. (1978) Diabetogenic effects of pregnancy in sows on plasma glucose and insulin release. *J. Anim. Sci. 46*, 1694–1700.

[54] Kemp, B., Soede, N. M., Vesseur, P. C., Helmond, F. A., Spoorenberg, J. H., and Frankena, K. (1996) Glucose tolerance of pregnant sows is related to postnatal pig mortality. *J. Anim. Sci. 74*, 879–885.

[55] Bouillon-Hausman, D., Kasser, T. R., Seerley, R. W., and Martin, R. J. (1986) Studies of gestational diabetes using the pig as a model in *Swine in Biomedical Research* (Tumbleson, M. E., Ed.) Vol. 1, pp. 561–572. Plenum Press, New York.

[56] Aaron, D. K., and Hays, V. W. (1991) Statistical techniques for the design and analysis of swine nutrition experiments in *Swine Nutrition* (Miller, E. R., Ullrey, D. E., and Lewis, A. J., Eds.) pp. 605–622. Butterworth-Heinemann, Stoneham, MA.

[57] Lindemann, M. D., Harper, A. F., and Kornegay, E. T. (1995) Further assessments of the effects of supplementation of chromium from chromium picolinate on fecundity in swine. *J. Anim. Sci. 73 (Suppl. 1)*, 185.

[58] Campbell, R. G. (1998) Chromium and its role in pig meat production in *Proc. Alltech's Fourteenth Ann. Symp.* (Lyons, T.P., and Jacques, K. A., Eds) pp. 229–237. Nottingham University Press, Loughborough, England.

[59] Charraga, A. S., and Cuaron, I. J. A. (1998) Chromium picolinate and cane molasses in breeding gilts diets in *Proceedings of the XXXIII Annual National Livestock Research Meeting, Queretaro, MX, October 27–31*, p. 117.

[60] Hagen, C. D., Lindemann, M. D., and Purser, K. W. (2000) Effect of dietary chromium tripicolinate on productivity of sows under commercial conditions. *Swine Health Prod. 8*, 59–63.

[61] Lindemann, M. D., Hall, R. E., and Purser, K. E. (2000) Use of chromium tripicolinate to improve pigs born alive confirmed in multiparous sows in *Proceedings of the American Association of Swine Practitioners. 31st Annual Meeting*, pp.133–137, March 11–14, Indianapolis, IN.

[62] Lindemann, M. D., Carter, S. D., Chiba, L. I., Dove, C. R., Lemieux, F. M., and Southern, L. L. (2004) A regional evaluation of chromium tripicolinate supplementation of diets fed to reproducing sows. *J. Anim. Sci. 82*, 2972–2977.

[63] Perez-Mendoza, V. G., Cuaron, J. A., Rapp, C. J., and Fakler, T. M. (2003) Lactating and rebreeding sow performance in response to chromium-L-methionine. *J. Anim. Sci. 81 (Suppl. 2)*, 71.

[64] Bortolozzo, F. P., Pinheiro Machado, I., Wentz, I., Nagae, R., and Penz, A. M., Jr. (1998) Effect of chromium picolinate on swine reproduction. I. Influence on number of ovulations, number of viable embryos and embryo survival in *Proceedings of the 15$^{th}$ International Pig Veterinary Society Congress*, p. 79, Birmingham, England.

[65] Jovanovic, L., Gutierrez, M., and Peterson, C. M. (1999) Chromium supplementation for women with gestational diabetes mellitus. *J. Trace Elem. Exp. Med. 12*, 91–97.

[66] Woodworth, J. C., Tokach, M. D., Nelssen, J. L., Goodband, R. D., Dritz, S. S., Koo, S. I., Minton, J. E., and Owen, K. Q. (2002) Influence of dietary carnitine and/or chromium on blood parameters of gestating swine in *Kansas State University Swine Day 2002 Reports*, pp. 23–47.

[67] Britt, J. H., Armstrong, J. D., and Cox, N. M. (1988) Metabolic interfaces between nutrition and reproduction in *Proceedings of 11th International Congress of Animal Reproduction and Artificial Insemination*, pp.117–125, Dublin, Ireland.

[68] Cox, N. M., Stuart, M. J., Althen, T. G., Bennett, W. A., and Miller, H. W. (1987) Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. *J. Anim. Sci. 64*, 507–516.

[69] Whitley, N. C., Moore, A. B., and Cox, N. M. (1998) Comparative effects of insulin and porcine somatotropin on postweaning follicular development in primiparous sows. *J. Anim. Sci. 76*, 1455–1462.

[70] Ramirez, J. L., Cox, N. M., and Moore, A. B. (1997) Influence of exogenous insulin before breeding on conception rate and litter size of sows. *J. Anim. Sci. 75*, 1893–1898.

[71] Whitley, N. C., Thomas, M., Ramirez, J. L., Moore, A. B., and Cox. N. M. (2002) Influences of parity and level of feed intake on reproductive response to insulin administration after weaning in sows. *J. Anim. Sci. 80*, 1038–1043.

[72] *Merck Veterinary Manual* (9th edn) (2005). Pp. 2586–2587. Merck & Co., Inc., Whitehouse Station, NJ.

[73] Bunting, L. D., Fernandez, J. M., Thompson, D. L., Jr., and Southern, L. L. (1994) Influence of chromium picolinate on glucose usage and metabolic criteria in growing Holstein calves. *J. Anim. Sci. 72*, 1591–1599.

[74] Depew, C. L., Bunting, L. D., Fernandez, J. M., Thompson, D. L., Jr., and Adkinson, R. W. (1998) Performance and metabolic responses of young dairy calves fed diets supplemented with chromium tripicolinate. *J. Dairy Sci. 81*, 2916–2923.

[75] Kegley, E. B., Galloway, D. L., and Fakler, T. M. (2000) Effect of dietary chromium-L-methionine on glucose metabolism of beef steers. *J. Anim. Sci. 78*, 3177–3183.
[76] Chang, X., and Mowat, D. N. (1992) Supplemental chromium for stressed and growing feeder calves. *J. Anim. Sci. 70*, 559–565.
[77] Moonsie-Shageer, S., and Mowat, D. N. (1993) Effect of level of supplemental chromium on performance, serum constituents, and immune status of stressed feeder calves. *J. Anim. Sci. 71*, 232–238.
[78] Mowat, D. N., Chang, X., and Yang, W. Z. (1993) Chelated chromium for stressed feeder calves. *Can. J. Anim. Sci. 73*, 49–55.
[79] Chang, X., Mowat, D. N., and Mallard, B. A. (1995) Supplemental chromium and niacin for stressed feeder calves. *Can J. Anim. Sci. 75*, 351–358.
[80] Mowat, D. N. (1997) Organic Chromium in *Animal Nutrition*. Chromium Books, Guelph, ON, Canada.
[81] Burton, J. L. (1995) Supplemental chromium: its benefits to the bovine immune system. *Anim. Feed Sci. Technol. 53*, 117–133.
[82] Chang, X., Mowat, D. N., and Spiers, G. A. (1992) Carcass characteristics and tissue-mineral contents of steers fed supplemental chromium. *Can. J. Anim. Sci. 72*, 663–669.
[83] Besong, S., Jackson, J. A., Trammell, D. S., and Akay, V. (2001) Influence of supplemental chromium on concentrations of liver triglyceride, blood metabolites and rumen VFA profile in steers fed a moderately high fat diet. *J. Dairy Sci. 84*, 1679–1685.
[84] Burton, J. L., Mallard, B. A., and Mowat, D. N. (1993) Effects of supplemental chromium on immune responses of periparturient and early lactation dairy cows. *J. Anim. Sci. 71*, 1532–1539.
[85] Yang, W. Z., Mowat, D. N., Subiyatno, A., and Liptrap, R. M. (1996) Effects of chromium supplementation on early lactation performance of Holstein cows. *Can. J. Anim. Sci. 76*, 221–230.
[86] Hayirli, A., Bremmer, D. R., Bertics, S. J., Socha, M. T., and Grummer, R. R. (2001) Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. *J. Dairy Sci. 84*, 1218–1230.
[87] Williams, C. C., Crochet, B. T., Bunting, L. D., Fernandez, J. M., and Stanley, C. C. (2004) Metabolic responses of periparturient Holstein cows and heifers supplemented with chromium picolinate. *Prof. Anim. Scientist 20*, 312–318.
[88] Villalobos, J. A., Romero, C., Tarrago, M. R., and Rosado, A. (1997) Supplementation with chromium picolinate reduces the incidence of placental retention in dairy cows. *Can. J. Anim. Sci. 77*, 329–330.
[89] Berradi, H., Cassy, C., Taouis, M., and Rideau, N. (2003) Partial cDNA cloning and immunodetection of a glucokinase in chicken liver and pancreas in *18th Intl. Diabetes Fed. Congress*, Abstract 1917, Paris, France. Aug 24–29.
[90] Rawdon, B. B. (1996) Avian gut and pancreatic endocrine cells: Developmental aspects in *VI International. Symposium on Avian Endocrinology, Abstracts, March 31–April 5*.
[91] Rideau, N. (1996) Insulin secretion in *VI International Symposium on Avian Endocrinology, Abstracts, March 31–April 5*.
[92] Cassy, S., Metayer, S., Crochet, S., Rideau, N., Collin, A., and Tesseraud, S. (2004) Leptin receptor in the chicken ovary: potential involvement in ovarian dysfunction of ad libitum-fed broiler breeder hens. *Repro. Biol. Endocrin. 2*, 72–79.
[93] Simon, J., Guillaumin, S., Chevalier, B., Derouet, M., Guy, G., Marche, G., Ricard, F. H., and LeClerq, B. (2000) Plasma glucose-insulin relationship in chicken lines selected for high or low fasting glycaemia. *Brit. Poult. Sci. 41*, 424–429.
[94] Steele, N. C., and Rosebrough, R. W. (1979) Trivalent chromium and nicotinic acid supplementation for the turkey poult. *Poult. Sci. 58*, 983–984.

[95] Steele, N. C., and Rosebrough, R. W. (1981) Effect of trivalent chromium on hepatic lipogenesis by the turkey poult. *Poult. Sci. 60*, 617–622.
[96] Ward, T. L., Southern, L. L., and Boleman, S. L. (1993) Effect of dietary chromium picolinate on growth, nitrogen balance and body composition of growing broiler chicks. *Poult. Sci. 72 (Suppl. 1)*, 37.
[97] Hossain, S. M., Barreto, S. L., and Silva, C. G. (1998) Growth performance and carcass composition of broilers fed supplemental chromium from chromium yeast. *Anim. Feed Sci. Technol. 71*, 217–228.
[98] Holoubek, J., Jankovsky, M., Arent, E., and Ledvinka, Z. (1997) Importance of organic form of chromium in turkey fattening. *Scientia Agricultura E Bohemica 28*, 147–159.
[99] Lien, T. F., Horng, Y. M., and Yang, K. H. (1999) Performance, serum characteristics, carcase traits and lipid metabolism of broilers as affected by supplemental chromium picolinate. *Br. Poult. Sci. 40*, 357–363.
[100] Lee, D. N., Wu, F. Y., Cheng, Y. H., Lin, R. S., and Wu, P. C. (2003) Effects of dietary chromium picolinate supplementation on growth performance and immune response of broilers. *Asian-Aust. J. Anim. Sci. 16*, 227–233.
[101] Wang, J., Du, R., Qin, J., Wang, S., Wang, W., Li, H., and Pang, Q. (2003) Effect of yeast chromium and L-carnitine on lipid metabolism of broiler chickens. *Asian-Aust. J. Anim. Sci. 16*, 1809–1815.
[102] Kim, Y. H., Han, I., Shin, I. Chae, B. J., and Kang, T. H. (1996) Effect of dietary excessive chromium picolinate on growth performance, nutrient utilizability and serum traits in broiler chicks. *Asian-Aust. J. Anim. Sci. 9*, 349–354.
[103] Kim, Y. H., Han, I. K., Choi, Y. J., Shin, I. S., Chae, B. J., and Kang, T. H. (1996) Effects of dietary levels of chromium picolinate on growth performance, carcass quality and serum traits in broiler chicks. *Asian-Aust. J. Anim. Sci. 9*, 341–347.
[104] Yeswanth, K. L. (1997) Effect of dietary chromium content on abdominal fat, cholesterol and high density lipoprotein content of broilers. Master of Science thesis. University of Agricultural Sciences, Bangalore, India.
[105] Kim, J. D., Han, I. K., Chae, B. J., Lee, J. H., Park, J. H., and Yang, C. J. (1997) Effects of Dietary chromium picolinate on performance, egg quality, serum traits and mortality rate of brown layers. *Asian-Aust. J. Anim. Sci. 10*, 1–7.
[106] Doerfler, R. E., Edens, F. W., McMurtry, J. P., Qureshi, M. A., Parkhurst, C. R., and Havenstein, G. B. (2000) Influence of biochrome on the response of metabolic hormones in PEMS-infected poults. *Poult. Sci. 79*, 661–668.
[107] Sahin, K., Sahin, N., and Küçük, O. (2003) Effects of chromium and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32 °C). *Nutr. Res. 23*, 225–238.
[108] Sahin, K., Sahin, N., Onderci, M., Gursu, F., and Cikim, G. (2002) Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. *Biol. Trace Elem. Res. 89*, 53–64.
[109] Sands, J. S., and Smith, M. O. (1999) Broilers in heat stress conditions: effects of dietary manganese proteinate or chromium picolinate supplementation. *J. Appl. Poult. Res. 8*, 280–287.
[110] Lien, T., Chen, S., Shiau, S., Froman, D. P., and Hu, C. Y. (1996) Chromium picolinate reduces laying hen serum and egg yolk cholesterol. *Prof. Anim. Scientist 12*, 77–80.
[111] Lien, T. F., Wu, C. P., and Lu, J. J. (2003) Effects of cod liver oil and chromium picolinate supplements on the serum traits, egg yolk fatty acids and cholesterol content in laying hens. *Asian-Aust. J. Anim. Sci. 16*, 1177–1181.
[112] Lien, T. F., Chen, K. L., Wu, C. P., and Lu, J. J. (2004) Effects of supplemental copper and chromium on the serum and egg traits of laying hens. *Br. Poult. Sci. 45*, 535–539.

[113] Xianlin, L., and Fanping, L. (1997) Effects of organic chromium on reduction of yolk cholesterol and alleviation of heat stress in laying hens. Fujian Agricultural University, PRC.

[114] Mathivanan, R., and Selvaraj, P. (2003) Influence of dietary chromium on egg production and quality parameters in layers. *Indian J. Poult. Sci. 38*, 51–53.

[115] Onderci, M., Sahin, N., Sahin, K., and Kilic, N. (2003) Antioxidant properties of chromium and zinc: in vivo effects on digestibility, lipid peroxidation, antioxidant vitamins, and some minerals under a low ambient temperature. *Biol. Trace Elem. Res. 92*, 139–150.

[116] Sahin, K., Sahin, N., and Kucuk, O. (2002) Effects of dietary chromium and ascorbic acid supplementation on digestion of nutrients, serum antioxidant status, and mineral concentrations in laying hens reared at a low ambient temperature. *Biol. Trace Elem. Res. 87*, 113–124.

[117] Sahin, N., and Sahin, K. (2001) Optimal dietary concentrations of Vitamin C and chromium picolinate for alleviating the effect of low ambient temperature (6.2 °C) on egg production, some egg characteristics, and nutrient digestibility in laying hens. *Vet. Med. Czech. 46*, 229–236.

[118] Kitchalong, L., Fernandez, J. M., Bunting, L. D., Southern, L. L., and Bidner, T. D. (1995) Influence of chromium tripicolinate on glucose metabolism and nutrient partitioning in growing lambs. *J. Anim. Sci. 73*, 2694–2705.

[119] Forbes, C. D., Fernandez, J. M., Bunting, L. D., Southern, L. L., Thompson, D. L., Gentry, L. R., and Chapa, A. M. (1998) Growth and metabolic characteristics of Suffolk and Gulf Coast Native yearling ewes supplemented with chromium tripicolinate. *Small Rumin. Res. 28*, 149–160.

[120] Olsen, Q. R., Rule, D. C., Field, R. A., Snowder, G. D., and Hu, C. Y. (1996) Dietary chromium-picolinate does not influence growth or carcass composition in feedlot lambs. *Sheep Goat Res. J. 12*, 22–24.

[121] Gentry, L. R., Fernandez, J. M., Ward, T. L., White, T. W., Southern, L. L., Bidner, T. D., Thompson, D. L., Jr., Horohov, D. W., Chapa, A. M., and Sahlu, T. (1999) Dietary protein and chromium tripicolinate in Suffolk wether lambs: effects on production characteristics, metabolic and hormonal responses, and immune status. *J. Anim. Sci. 77*, 1284–1294.

[122] Ott, E. A., and Kivipelto, J. (1999) Influence of chromium tripicolinate on growth and glucose metabolism in yearling horses. *J. Anim. Sci. 77*, 3022–3030.

[123] Socha, M. T., Ralston, S. L., Raub, R., Kegley, E. B., Swenson, C. K., Johnson, A. B., and Fakler, T. M. (1999) Metabolic effects of dietary chromium-L-methionine in horses and beef cattle in *Proceedings of the 10th International Symposium on Trace Elements in Man and Animal*, *May 2–7*, p. 312, Evian, France.

[124] Pagan, J. D., Rotmensen, T., and Jackson, S. G. (1995) The effect of chromium supplementation on metabolic response to exercise in Thoroughbred horses in *Proeedings of the 14$^{th}$ Equine Nutrition and Physiology Symposium, Jan 19–21*, pp. 96–101, Ontario, CA.

**Part II**

# Biochemical role(s) for chromium(III)

This page intentionally left blank

*Chapter 6*

# The transport of chromium(III) in the body: Implications for function

**Weiyue Feng**

Key Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics, Chinese Academy of Sciences

## ABSORPTION

Chromium(III) present in the diet could exist as inorganic form or as organic complexes. Generally, the absorption of dietary trivalent chromium is very small, between 0.5 and 2% [1], while the excess is excreted through urine and feces. However, it is suspected that the organic chromium, such as chromium from brewer's yeast, is more absorbable than inorganic one [2]. In fact, many studies have shown that the absorption coefficient of different chromium sources is variable [3] (see Table 1).

However, some absorption studies in humans showed that there was an inversely proportional absorption to the daily supply. At a daily dose of 10 μg, about 2% of the chromium was absorbed; while at the dose of 40 μg, chromium absorption decreased to 0.4–0.5% [10, 11]. Whereas studies in rats indicated that trivalent chromium could reach a consistent absorption and excretion regardless of previous diet history [10, 12, 13]. When supplied at dose higher than 40 μg, absorption remained constant at 0.4% [10]. Therefore, it was proposed that chromium homeostasis was maintained at the level of excretion rather than absorption, and chromium uptake by rats might be different from that of humans.

Dietary chromium is absorbed in the intestinal mucosa. An in vitro study of rats with $^{51}CrCl_3$ treatment, the middle section of the small intestine was shown as the most active segment for chromium absorption, then the ileum and the duodenum [14]. In humans, the site of Cr(III) absorption also includes the jejunum [15]. Some animal studies indicate that Cr(III) absorption in the small intestine is a nonsaturable process. It is absorbed by a nonmediated process of passive diffusion in the small intestine [6, 12]. However, results of in vitro studies using the isolated intestine from chromium-deficient rats showed that the percentage of Cr(III) absorption decreased with its concentration and increased in the incubation medium [16]. This behavior is not compatible with a simple diffusion process; it suggests a finite number of specific sites involved in the chromium absorption and the absorption mechanism is similar to facilitated diffusion [16]. Dowling

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

Table 1
Absorption of various chromium compounds (adapted from Ref. [3])

| Chromium source | Absorption (%) | Subject |
|---|---|---|
| Chloride | $0.9 \pm 0.2$ (4 hours)[4] | Rat |
| | 0.69 (mean range 0.3–1.3)[5] | Human |
| | $\sim 0.5$[6] | Rat |
| Nicotinate | $1.3 \pm 0.3$[4] | Rat |
| Picolinate | $1.1 \pm 0.3$[4] | Rat |
| | $2.8 \pm 1.14$ SD[7] | Human |
| Dinicotinic acid–diglycine–cysteine–glutamic acid complex | $0.6 \pm 0.1$[4] | Rat |
| Chromium from food | 1.8 (36.8 mg Cr/day)[8] | Human |
| | 2–3[9] | Human |
| Chromium from brewer's yeast | 5–10[5] | Human |

and coworkers [17] using a more physiologic surgical technique, called the double perfusion, which has been successful in studies of intestinal physiology, found that there was statistically significant linearity between chromium transport, retention, and uptake, and the luminal chromium concentration. Thus, it demonstrated that inorganic trivalent chromium was absorbed by a nonsaturable passive diffusion process.

The fate of dietary Cr(III) absorption can be significantly affected by many dietary and drug factors such as starch, ascorbic acid, minerals, oxalate, and amino acid intake.

In the studies in mice, animals fed 50% starch concomitantly with $^{51}CrCl_3$ were found to retain significantly higher concentrations of chromium in most tissues than those fed 50% glucose, sucrose, and fructose [18].

An intestinal absorption study of rats indicated that ascorbic acid addition could increase Cr(III) absorption [17]. A study in three women found that the ingestion with 100 mg ascorbic acid in conjunction with 1 mg $CrCl_3$ increased the absorption of chromium as much as 44, 177, and 348% by the patients, by measuring plasma levels [19].

Some chelating substances can combine with chromium and significantly influence the absorption of chromium in the gut tract. Oxalate was observed to significantly increase the concentrations of $^{51}Cr$ in blood, body tissues, and urine of rats after 24 hours of administration, whereas, both in vivo and in vitro studies demonstrated that phytate markedly decreased the absorption of chromium in the intestine of rats [14]. However, other chelators, such as citrate and ethylenediaminetetraacetic acid (EDTA), have not been demonstrated to have evident effects.

Some amino acids can prevent the precipitation of chromium at the basic pH of the intestine, thereby increasing chromium absorption. Histamine which could chelate chromium in the small intestine was reported to facilitate the absorption of chromium [12]. Tests with healthy elderly volunteers demonstrated that dietary nicotinic acid acts as a synergist with chromium absorption [20].

Some competition metals could drive chromium from its binding sites or form complexes with chromium and then modify its absorption. Absorption of $^{51}Cr$ was

increased in zinc-deficient rats and was reduced by oral administration of zinc [21]. This suggests that chromium and zinc share a common mechanism in the intestinal absorption. However, such result is not consistent with others. Anderson et al. [4] found no alteration in tissue levels of both copper and zinc when mice were fed a diet with 5000 ng Cr(III)/g body weight. Besides zinc, vanadium, and iron were all found to decrease the absorption of chromium [22, 23]. One experiment on chicks observed that large quantities of chromium prevented the reduced growth and death of chicks with high dietary supplementation of vanadium [22]. Iron-deficient rats absorbed more chromium than iron-replete rats. The administration of iron in rats could inhibit the absorption of chromium [23]. This investigation demonstrates that chromium and iron have a similar transport and absorption mechanism. In an in vitro rat study, manganese and calcium all showed to depress intestinal absorption of chromium at levels of 100-fold that of chromium, while in the case of titanium, 10 times titanium concentrations of chromium was shown to inhibit the absorption [24].

Additionally, Cr(III) absorption can be affected by plasma proteins, such as transferrin and albumin [17]. These plasma proteins act as important transport proteins of chromium which will be discussed later.

Other physiological factors can influence chromium absorption as well. Medications can enhance or impair chromium absorption. Rats orally administered with 40 mg aspirin, a nonsteroidal anti-inflammatory drug (NSAID) and prostaglandin inhibitor, exhibited markedly enhanced absorption, tissue retention, and urinary excretion of $^{51}Cr$ [25]. Intraperitoneal injection of indomethacin 5 mg/kg body weight significantly increased $^{51}Cr$ in blood, tissues, and urine of rats, whereas dosing with 16,16-dimethylprostaglandin E2, a prostaglandin E2 analogue, decreased $^{51}Cr$ absorption. These results demonstrated that blocking the synthesis of prostaglandins enhances chromium absorption [26].

## TRANSPORTATION

### Transferrin

In pH 7.35, human plasma proteins were found to strongly bind with $Cr^{3+}$, while lipemia of plasma decreases the binding of $Cr^{3+}$ to the proteins [27]. Results from in vitro and in vivo investigations have demonstrated that of all serum proteins, the ferric iron transport protein, transferrin, binds almost all the chromium administered [23, 28–30]. Hopkins and Schwarz [23] reported that more than 99% of the chromium in the blood was associated with noncellular components when $CrCl_3$ was gastrointestinally administrated to rats. Further, they stated that approximately 90% of chromium in the serum was associated with the blood $\beta$-globulin fractions, while 80% immunoprecipitates were with transferrin [23]. A similar percentage data of serum chromium associated with transferrin was also reported by Sayato and his colleagues [28] after the intravenous injection of $CrCl_3$ to rats. In vivo oral administration of chromic ions to rats exhibited the chromic ions in the fractions of transferrin as well [29].

When excessive amounts of chromium are given, other protein fractions such as albumin, $\gamma$- and $\beta$-globulins, and lipoproteins in blood can also bind the element [23]. In in vitro $^{51}Cr$-labeled studies, added chromium in plasma was shown mainly to bind

to transferrin and then of a less degree to albumin and some degradation products [30]. Such observation distinguishes the behavior of chromium from that of Cu, Se, Zn, and so on, all of which were shown to bind to albumin first. Since all the evidence indicates the great affinity of chromium for transferrin, it has been assumed that transferrin is involved in chromium transport.

Transferrin, the ~80 kDa metal-binding globulin of blood protein, possesses two specific binding sites, A and B, with different affinities for two equivalents of ferric iron at neutral and slightly basic pH levels. Generally, in humans, the coordinate sites of transferring is loaded only about 30% with iron on average, allowing it has the possibility to bind and carry other metal ions [29]. The electron paramagnetic resonance (EPR) spectra study of $Cr^{3+}$-transferrin indicated that $Cr^{3+}$ was bound to site B at pH 6.0 [31]. Spectrophotometric titration of each individual amino acid located in the iron binding site of transferrin revealed that tyrosine might be the most suitable ligand for the binding of chromium to transferrin [32]. However, the binding of $Cr^{3+}$ to apo-transferrin appears to be pH dependent. In fact, with the results of protein's ultraviolet spectrum and EPR study, Cr(III) is demonstrated to readily bind to the two ferric ion-binding sites [31–35]. The two binding sites can be distinguished by EPR, and only chromic ions at the A site or the C-lobe site can be displaced by $Fe^{3+}$ at near neutral pH [31, 36]. Below pH 6, only the B site or the N-lobe site binds Cr [37]. Sun and coworkers [38] calculated the binding constants of chromium to transferrin as follows: $K_1 = 1.42 \times 10^{10} M^{-1}$, $K_2 = 2.06 \times 10^5\ M^{-1}$, which gives the overall effective binding constant as $2.92 \times 10^{15}\ M^{-2}$. This binding constant for chromic ions at the two sites is shown to differ at five orders of magnitude, which is consistent with the previous research of $Cr^{3+}$ having the site-selective binding in transferrin.

At high concentrations of either chromium or iron, such as hemochromatosis, $Cr^{3+}$ and $Fe^{3+}$ were found to act as antagonist with each other [35]. The interaction of iron and chromium is thought to be linked to the shared binding sites of transferrin. An in vivo study showed that the serum levels of iron, total iron binding capacity (TIBC), and ferritin were reduced following daily administration of chromium (1 mg/kg) for 45 days to rats [32]. Hemochromatosis is an iron storage disease in humans characterized by highly saturated transferrin levels and sometimes by diabetes. Sargent et al. [39] first proposed the theory that increased iron stores due to hemochromatosis might result in the exclusion of chromium from metabolic binding sites and then induce to diabetic symptoms. They found that patients with hemochromatosis had significantly less plasma chromium than iron-removal patients. When the saturation of transferrin with iron increases over 50% in blood, iron competes with chromium binding and affects chromium transportation [39]. This theory is further supported by another study of patients with hemochromatosis who were found to have significantly higher excretion of the unbound plasma chromium as well as have a smaller blood pool of chromium due to the saturation of transferrin by iron [40].

Extensive clinical, epidemiologic, and basic studies suggest that excessive tissue iron stores may contribute to the occurrence and complications of diabetes mellitus [41]. Epidemiologic studies have repeatedly shown positive correlation between levels of serum ferritin and those of fasting glucose, insulin, and glycosylated hemoglobin. Iron reduction therapy in hereditary hemochromatosis and transfusional iron overload is associated with improved glucose tolerance and reduced incidence of secondary diabetes [41].

Therefore, the reports on the effects of insulin on iron transport and the relationship between hemochromatosis and diabetes suggest that transferrin may actually be the major physiological chromium transport agent.

**Low-molecular-weight chromium-binding substance (chromodulin)**

Although it has already known that the intake chromium is transported by the fractions of serum proteins and is rapidly taken up by other tissues, however, until 1980s, little was known about the intrinsically chemical form of chromium in the organic tissues.

In 1981, Wada, Yamamoto, and Ono [42] successfully isolated a high chromium-containing oligopeptide named "low-molecular-weight chromium-binding substance" (LMWCr) in the liver cytosol of mice after intraperitoneal injection of potassium dichromate. Meanwhile, similar substance was also found in mouse plasma, urine, and feces. Subsequently, LMWCr was isolated and purified from dog liver [43], rabbit liver [44], bovine colostrums and liver [45, 46], porcine kidney and kidney powder [47], which suggested that LMWCr is extensively present in mammals. This natural chromium-containing oligopeptide is identified as an anionic, organic chromium compound with a molecular weight of 1500 contains glutamic acid, glycine, and cysteine as the predominant amino acids [45, 46] and is later called "chromodulin" [48]. Spectroscopic investigation suggests that chromium tightly bind four equivalents of chromic ions in a multinuclear assembly (holochromodulin) like that of calmodulin [48].

Animal study indicated that high amounts of LMWCr could soon occur in the liver cytosol after chromium administration [49]. However, the content of LMWCr decreased to a trace amount within several days [42, 49]. Yamamoto and coworkers [49] investigated the LMWCr distribution in organs of mice and found that large amount of chromium soon accumulated in the liver and the kidneys, among them 72% chromium accumulated as LMWCr in the liver, and 84.4% as LMWCr in the kidney 2 hours after chromium treatment. Approximately 55% chromium of total chromium presented as LMWCr in the spleen, intestines, and testicles. In the meantime, 22–25% chromium presented as LMWCr in the brain and plasma. These results suggest that LMWCr widely exist in animal tissues and is mainly bound with chromium after chromium administration [49].

Low-molecular-weight chromium-binding substance is thought to exist originally in organ as chromium-free apo-LMWCr since the formation of LMWCr is soon after chromium incubation with organic cytosol and no inductive formation of LMWCr could be obtained though excessive amounts of chromium is added [44, 46, 49].

In an in vitro study under physiological pH conditions in the bloodstream, Cr(III) as $CrCl_3$ bound to LMWCr was slightly more than those bound to transferrin and twice as much as those bound to albumin [49]. The comparative affinity of Cr(III) for LMWCr and the plasma proteins decreases in the following order: LMWCr, transferrin, albumin. High amount of Cr(III) was found to transfer from LMWCr to transferrin and vice versa, and from albumin to transferrin. Nevertheless, little amounts of Cr(III) was found to transfer from LMWCr to albumin and vice versa or from transferrin to albumin [49]. Therefore, when chromium is transferred to organs, chromium could react with transferrin as a form of LMWCr.

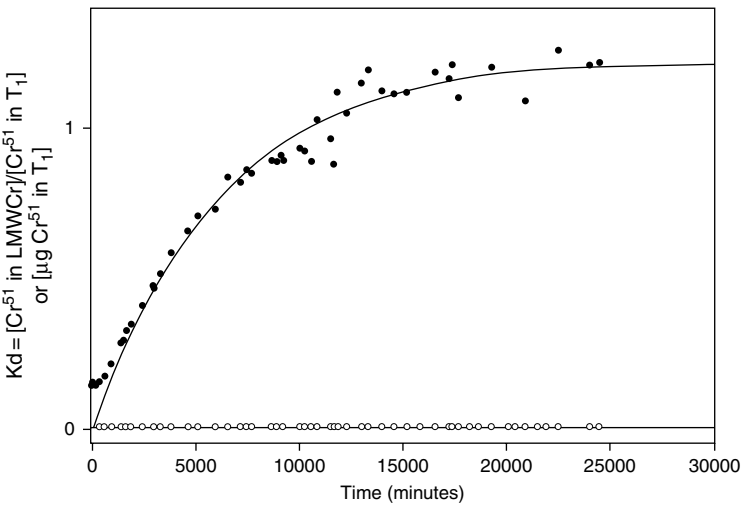



Fig. 1. Transfer of chromium between LMWCr and transferrin as a function of time. *Open circles*, µg $Cr^{3+}$ transferred from LMWCr to apotransferrin; *solid circles*, distribution constant for $Cr^{3+}$ between $Cr_2$-transferrin and apo-LMWCr. Reproduced from Ref. [38] with the permission of the copyright holder.

Recently, Sun and coworkers [38] determined the ability of apo-LMWCr bind to chromium by a variation of the equilibrium dialysis method. Their study indicates that four chromic ions bind to apo-LMWCr in a highly cooperative fashion ($n = 3.47$) with a binding constant of $1.54 \times 10^{21}$. In this in vitro study, apo-LMWCr with its full complement of four chromium was not shown to release its chromium to apo-transferrin over more than 25,000 minutes incubation (Fig. 1). However, over the same period, about half of the chromium was observed to transfer from $Cr_2$-transferrin to apo-LMWCr. These results demonstrate that chromium could readily transfer from transferrin to apo-LMWCr at near neutral pH but the opposite transfer is seldom, which is somewhat different from the previous work of Yamamoto et al. [49]

## CHROMIUM TRANSPORT TO ORGANS

The distribution studies involving the intravenous and intraperitoneal administration of $^{51}CrCl_3$ to human and to animals have shown that Cr(III) is principally accumulated in liver, moderately accumulated in kidneys, spleen, and muscle, while it is also spread in many other organs, such as heart, pancreas, lung, bone, and brain [12, 40, 50–58].

Mertz et al. [12] found that the absorption and excretion of $^{51}Cr$ intravenous injection was independent of the injected amount and of the nutritional status of the animals. They described the body retention of chromium in terms of three compartments with three half-lives ($T_{1/2}$) as 0.5, 5.9, and 83.4 days (with 72 days of observation). Similar results were reported in subsequent studies as well [54–56].

Onkelinx [55] studied the metabolism of Cr(III) in rats of different ages after intravenous injection of $^{51}CrCl_3$ in trace amounts. A three-compartment model was described depending on the plasma disappearance curve. The data supports a hypothesis that specific transport characteristics exist in tissues that may regulate the biological role of chromium. Their data show that the extracellular chromium is in equilibrium with two tissue compartments of different rates of chromium exchanges and chromium can also enter nonexchangeable tissues.

The study of Jain et al. [56] supports the hypothesis of Onkelinx which indicates that a complex type of exchange involves both a rapidly exchanging Cr pool and an "inner" Cr pool with "sink-like" characteristics. Chromium deprivation of moderate degree reduces serum Cr and tissue exchangeable Cr pools but do not change total tissue Cr, Cr distribution, or glucose tolerance. Chromium supplementation and Cr overload increase serum Cr and total, exchangeable, and nonexchangeable tissue Cr pools but do not alter Cr distribution.

Similar results were reported in human study as well. Lim et al. [40] have studied the distribution and kinetics of intravenous $^{51}$Cr-labeled Cr(III) in six human subjects. The high counts of $^{51}$Cr were found in the liver, spleen, soft tissues, and bones. The kinetic study indicated that the data were fit to a model consisting of a plasma pool in equilibrium with fast ($T_{1/2} = 0.5$–12 hours), medium (1–14 days), and slow (3–12 months) compartments. They proposed a centralized multicompartment physiological model for Cr(III) transport. The model considers plasma chromium to be the central compartment, consisting of two subcompartments called BB (Cr bound to proteins) and BF (free Cr), and peripheral compartments, including the liver and spleen. The model exhibits exchanges between BB and BF. The liver and spleen are assumed to consist of a compartment that accumulates chromium with a high rate constant, and another compartment that stores chromium and releases it with a much slower rate constant.

When chromium transport to tissues, it could bind with both high-molecular-weight compounds (HMWCr) and low-molecular-weight substance (LMWCr) [28, 49, 59]. Sayato et al. [28] have comprised the metabolic fate of $^{51}CrCl_3$ and $^{51}CrO_4^{2-}$ in the rat liver and blood by S-200 gel filtration after the oral and the intravenous administration. A significant difference was observed in the rat blood that trivalent chromium possessed a high binding activity for transferrin in plasma, while hexavalent chromium was permeable into red cells and bound with hemoglobin. In hepatocyte cytosol, the bands from different chromium sources were significantly different as well. $^{51}$Cr from $CrCl_3$ was primarily bound to high-molecular-weight proteins.

Feng et al. [59] studied the distribution patterns of chromium-containing proteins in the hepatocyte cytosol and serum of the normal and the diabetic rats after a single intravenous injection of enriched stable isotopic Cr-50 ($^{50}CrCl_3$) tracer solution. The results indicated that chromium was mainly bound with a high-molecular-weight protein either in liver cytosol or in serum samples of both the normal and the diabetic rats. Unfortunately, both Sayato et al. [28] and Feng et al. [59, 60] have not identified the high-molecular-weight chromium-containing compounds. However, further investigation of chromium-containing proteins in subcellular fractions of rat liver indicated that chromium in nuclei, microsome, and cytosol of liver were principally combined with high-molecular-weight proteins [60]. According to the relative molecular mass, this HMWCr is assumed to be transferrin. Therefore, comparing the results of $^{51}CrCl_3$

distribution in animals with the above studies suggest that Cr-transferrin have been observed in all of the studies.

Since transferrin is considered as the major physiological chromium transport agent and its function may be disturbed by insulin [61], recently the uptake of $Cr^{3+}$ from bloodstream to tissues and its excretion affected by glucose and insulin have been further studied [38, 62–64]. Morris et al. [62] in their in vitro investigation indicated that there was difference in chromium binding between insulin-sensitive tissues (liver, adipose tissue, muscle, etc.) and insulin-insensitive tissues and such binding was significantly enhanced by glucose in insulin-sensitive tissues. This study supports the hypothesis that, in vivo, chromium translocates from the blood compartment to insulin-sensitive tissues. Recently, Clodfelder et al. [64] in the in vitro study of rats demonstrated that the addition of insulin resulted in a significant increase in the incorporation of chromium into liver, indicating that the transfer was insulin sensitive.

Furthermore, Clodfelder and coworkers [64] have well demonstrated the role of transferrin undertaking the transport of chromium from blood to tissues and the effects of insulin on chromium incorporation. They injected $^{51}Cr_2$-transferrin into rats with or without insulin added, 2 hours later chromium from transferrin was found to incorporate into all examined tissues. Insulin treatment resulted in a significant increase in the transfer of chromium into tissues, especially in the liver and kidneys (Fig. 2). The concentration of chromium significantly increased after insulin treatment. All these results further demonstrate that transferrin is primarily responsible for chromium transport to tissues. Subsequent gel filtration research of hepatocyte cytosol showed that the increase in $^{51}Cr$ concentration was associated almost exclusively with chromium binding to transferrin after insulin addition.

Recently, Cr-loaded transferrin has been demonstrated to transport chromium in vivo [65]. When $^{51}Cr$-labeled transferrin was intravenously injected into the bloodstream, a rapid and insulin-sensitive movement of chromium into the tissues as Cr-transferrin was observed. The results showed that more than 50% of the chromium was transported into the tissues within 30 minutes after injection. In this study, insulin appeared to accelerate the processing of $^{51}Cr$ by the tissues. The disappearance of $^{51}Cr$-transferrin from the blood in the beginning time after injection was greater in the rats receiving insulin than those not receiving insulin. This indicates that insulin treatment has the effect of lowering retained chromium in tissues. In this study, the chromium decrease in tissue with time was mirrored by chromium increase in urine, which showed a rapid movement from $^{51}Cr$-transferrin to the tissues and to the urine. More important observation was that insulin not only increased the transfer of $^{51}Cr$ from transferrin to the tissues but also resulted in much greater movement of $^{51}Cr$ into LMWCr. When insulin was administered, the amount of LMWCr in the cytosol rose for the first 360 minutes and then decreased compared with the result of absence of insulin treatment that the amount of LMWCr rose for the first 120 minutes and then decreased steadily. The hepatocyte cytosol study showed that the Cr-transferrin peak presented first, then the LMWCr peak appeared at an appreciably delayed time, suggesting that insulin effect the processing of chromium removal from transferrin and binding to LMWCr.

However, the transport of Cr-transferrin affected by insulin exhibits far more complex behavior as the curve of the blood plasma levels of $^{51}Cr$ cannot be explained by a simple three-exponential decay which was previously reported [12, 40, 55, 56].

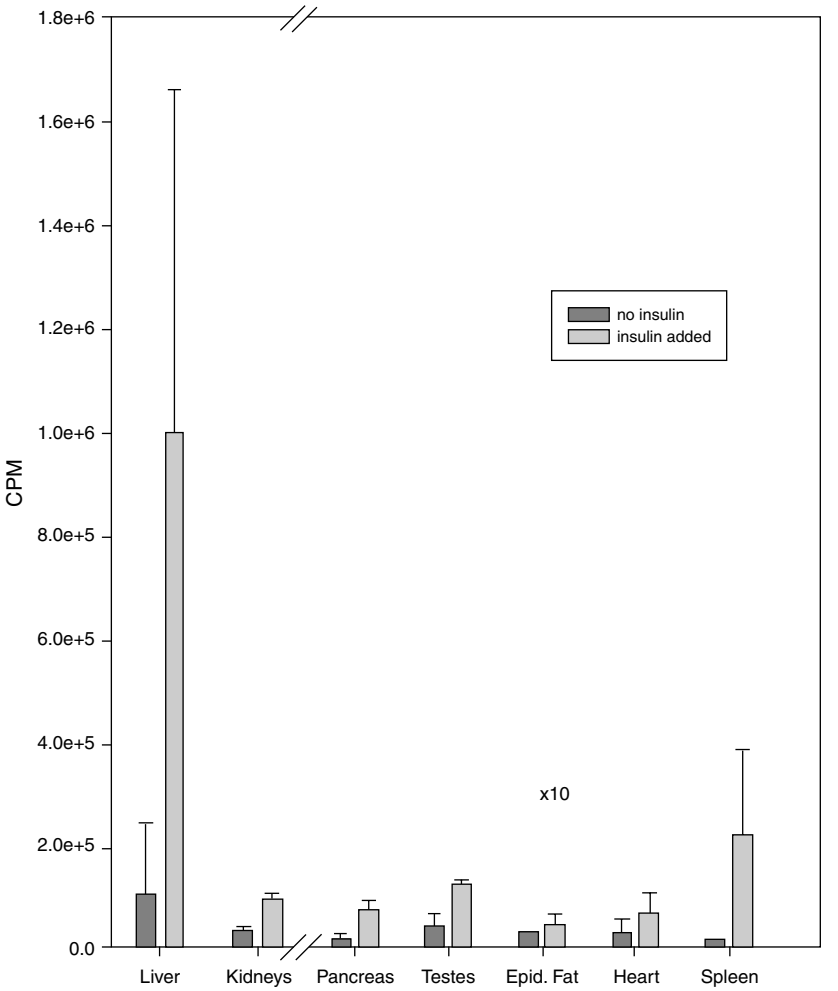



Fig. 2. Transfer of Cr from $Cr_2$-transferrin to tissue in vivo. Differences in Cr content between each tissue type with and without added insulin were statistically significant ($P < 0.05$). Reproduced from Ref. [64] with the permission of the copyright holder.

One reason for this failure is assumed to be the behavior of insulin because, in the presence of insulin, the rapid loss of plasma $^{51}Cr$ was followed by a small increase in the $^{51}Cr$ content. Thus, the behavior of chromium appears more complex than rate-limiting exchanges of chromium and rapid loss of chromium in the urine. Therefore, a constant $^{51}Cr$ background and an exponential loss of $^{51}Cr$ are suggested to fit the behavior of the plasma $^{51}Cr$ levels in both with presence and absence of insulin when Cr-transferrin is administrated. This result also suggests that the metabolism of Cr-containing species may be more complex than simple chromic salts and insulin or carbohydrate intake may affect chromium metabolism.

## EXCRETION

Absorbed chromium is excreted principally in the urine, and in small quantities in the hair, sweat, and bile [54, 58, 66]. At least 80% of the absorbed chromium is eliminated via the kidneys [67]. The urinary chromium loss of humans coincides with the amount of chromium lost from the blood plasma [61].

Stresses due to high sugar diets, strenuous exercise, physical trauma, and pregnancy and lactation have been shown to enhance chromium losses [68]. Kozlovsky et al. [69] have shown that subjects who consumed high sugar (35% of total calories) generally had increased urinary chromium excretion compared with when they consumed only 15% of total calories from simple sugars. Morris et al. [62] reported in in vitro research that insulin treatment affected the transfer of chromium from the blood plasma and increased excretion in urine. They demonstrated that chromium reduced in plasma and increased in urine in response to sugar intake regulated by insulin action. Similar results were reported by Clodfelder and coworkers [64] who indicated that insulin treatment increases chromium concentrations in the urine of rats.

A supplementation study of inorganic chromium (chromic chloride) in volunteers has stated that chromium concentration correlated with a glucose challenge but not with serum glucose, insulin, nor lipid parameters nor age nor body weight [70]. Similar results were obtained from urine samples obtained from subjects during chromium supplementation [71]. These results suggest that urinary chromium excretion does not appear to be a meaningful indicator of chromium status but is a meaningful indicator of chromium intake [70, 71].

The detailed mechanism of renal chromium metabolism is not clearly known. Previous studies suggest that 5–40% of plasma Cr(III) is ultrafilterable and that 60–95% of filtered chromium is reabsorbed in the renal tubule. An increased chromium excretion may result from a glucose challenge [72]. A study of the effect of free-water diuresis on antidiuretic hormone (ADH)-deficient rat was reported having little effect on body $^{51}Cr$ retention or relative tissue distribution of $^{51}Cr$ but did induce 25–40% increases in serum $^{51}Cr$ concentrations. This result seems to support the mechanism of proximal tubule reabsorption of chromium [73]. However, Donaldson et al. [74] indicated that the urinary chromium was equal to the filtered chromium, so he suggested that the predominant mechanism would be glomerular filtration without reabsorption.

Finally in the 1980s, LMWCr was isolated and purified from mammals, and a third mechanism of renal metabolism was suggested. It was found in the urine of humans and rats that chromium was apparently bound with LMWCr [42, 61]. Low-molecular-weight chromium-binding substance exhibits higher rates of urinary excretion and renal clearance in animals, accompanied by lower rates of renal tubular reabsorption and kidney and liver accumulation than inorganic chromium compounds [44]. Low-molecular-weight chromium-binding substance exists in plasma, urine, and feces [43]. Therefore, the third hypothesis does not involve a classical renal mechanism, but a one of binding.

Insulin-dependent diabetics excrete a larger proportion of intravenously administered $^{51}Cr$ than healthy people do [66]. Similar results were obtained from insulin-dependent diabetic children who excreted a much greater amount of chromium in their urine than normal children did [75]. Feng et al. [58] observed that the diabetic rats

excreted more amounts of chromium as low-molecular chromium-containing substance in urine than the healthy rats did.

Clodfelder et al. [64] in their study support a direct link between carbohydrate metabolism and LMWCr. They found that the increased chromium concentrations in the urine 2 hours after insulin treatment compared to the concentrations in controls were accompanied by a loss of chromium from the blood plasma of rats treated with insulin. Chromatographic study has revealed that chromium from $Cr_2$-transferrin appears as a single form in urine. Subsequently, the urine chromium was demonstrated to be in the form of LMWCr. Thus, increased blood insulin levels results in increased urinary LMWCr.

Recently, in a Cr-loaded transferrin transportation study [65], it was found that injection of the labeled $^{51}$Cr-transferrin and insulin resulted in a several fold increase of urinary $^{51}$Cr loss. Further chromatographic investigation showed that only one radio-labeled species appeared in the urine. This single $^{51}$Cr-containing species was identified as LMWCr (chromodulin). Thus, transferrin in an insulin-dependent fashion can transfer chromium to LMWCr where it is excreted in the urine. The removal of $^{51}$Cr from the blood is faster than the appearance of $^{51}$Cr in the urine; the lag in time indicates that the Cr-transferrin in the blood and chromium in the urine are not in direct equilibrium and that intermediates in the transport of chromium must be involved.

## CHROMIUM–TRANSFERRIN–APO-LMWCR (CHROMODULIN) TRANSPORT MODEL

Based on the current results, a model for the absorption and transportation of chromium from transferrin in the blood plasma to tissues then excretion in the urine has been preliminarily established.

Since transferrin is considered as the major physiological chromium transport agent and its function may be disturbed by insulin [61], glucose and insulin alteration could therefore disturb chromium metabolism significantly.

Insulin could dramatically stimulate plasma membrane recycling of transferrin. Upon insulin stimulation, insulin-responsive vesicles rapidly fuse with the plasma membrane and deliver all their cargo proteins, including transferrin to the cell surface [76]. Low-molecular-weight chromium-binding substance is maintained inside insulin-dependent cells almost entirely in its metal-free apo-form. The mobilizable chromium could readily transfer from $Cr_2$-transferrin to apo-LMWCr at near neutral pH in the blood. In response to the elevated blood insulin level, LMWCr processes a complicated movement of chromium, including generation of LMWCr in its active form as a multinuclear (probably tetranuclear) chromic assembly (holochromodulin) [77], in insulin-dependent cells [62, 63]. When insulin levels drop and receptor activity diminishes, holochromodulin is rapidly expelled by cells to the blood and excreted in the urine [77].

This model establishes a clear pathway of transport of chromium starting from transport by transferrin from the bloodstream into the tissues, followed by release and processing in the tissues to form “LMWCr (chromodulin)”, excretion of “LMWCr (chromodulin)” into the bloodstream, and rapid clearance of chromium as “LMWCr (chromodulin)” into the urine (Fig. 3).

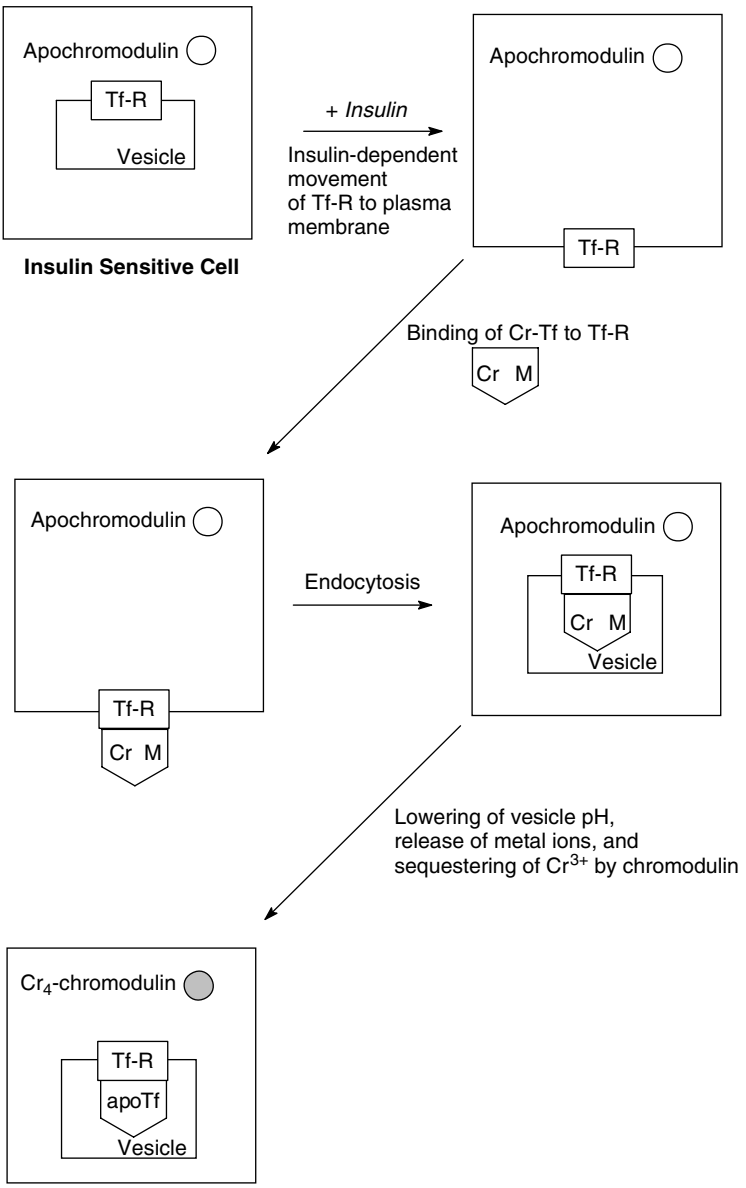



Fig. 3. Proposed mechanism for the movement of chromium from blood to chromodulin. In response to increases in plasma insulin concentrations, transferrin-receptor (Tf-R) in insensitive cells migrates from vesicles to the plasma membrane. Transferrin (pentagon), which contains two bound metal ions (in this case one chromic ion and one other metal cation (M)), binds to the receptor and is internalized by endocytosis. The pH of the resulting vesicle is reduced by ATP-driven proton pumps, resulting in the release of the metal ions from transferrin. Chromium released from multiple transferrin molecules is sequestered by apochromodulin (open circle) to produce chromium-loaded chromodulin (dark circle). Reproduced from Ref. [77] with the permission of the copyright holder.

In adult-onset diabetics, whose blood chromium levels are reduced and urinary chromium losses are increased, this transport system may be exceeding normal operation.

## DISCUSSION

Currently, the exact mechanism of transport of Cr(III) in mammals is still unclear. Previously, pharmokinetic studies of intravenous injection of $CrCl_3$ have identified three exchangeable pools of tissue chromium with the plasma chromium. One is exchangeable with a half-life ($T_{1/2}$) of less than one day (0.5–12 hours), the second is exchangeable over days (1–14 days) and the third is exchangeable over months (3–12 months) [12, 40, 54–56].

However, one recent study indicated that during the simultaneous intravenous injection of $Cr_2$-transferrin and insulin to rats, failure of the above model occurred [64]. In this case, a rapid decrease of $^{51}Cr$ is followed by a little increase of $^{51}Cr$ along the time. Two $^{51}Cr$-containing unidentified complexes were found in the plasma with and without insulin treatment. One presented maximal at the beginning point of time after injection, another elevated with time. Nevertheless, when $CrCl_3$ is intravenously injected, no such phenomenon has been observed. Thus, it demonstrated that the behavior of Cr-containing species or dietary chromium may be far more complex than simple chromic salts. Additional experiments are necessary to determine the Cr-containing species and rates of exchange in different organs and tissues so that a more sophisticated pharmokinetic model of chromium transport can be developed.

The chromium that transports to tissues and is lost within 2 hours after injection as LMWCr (chromodulin) in urine may represent the fast exchangeable pool in the above models and has been partly elucidated. However, more work will be necessary to determine the forms in which chromium, representing the other two pools, is stored in cells, bones, or other materials of the body.

Other questions such as how Cr(III) is transferred from acidified transferrin-containing endosomes to the cytosol and nucleus to bind to LMWCr (chromodulin) need to be elucidated.

## REFERENCES

[1] World Health Organization, International Atomic Energy Agency, Food and Agricultural Organization of the United Nations (1996) Trace Elements in *Human Nutrition and Health*. World Health Organization, Geneva.

[2] Mertz, W. (1975) Effects and metabolism of glucose tolerance factor. *Nutr. Rev. 33*, 129–135.

[3] Davis, W. L., and Steven, M. P. (2002) The safety and efficacy of high-dose chromium. *Alt. Med. Rev. 7*, 218–235.

[4] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Gautschi, K. (1996) Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. *J. Trace Elem. Exp. Med. 9*, 11–25.

[5] Mertz, W., and Cornatzer, W. E. (1971) *International Symposium on the Newer Trace Elements in Nutrition: Newer Trace Elements in Nutrition*. M. Dekker, New York, NY.

[6] Donaldson, R. M., Jr., and Barreras, R. F. (1966) Intestinal absorption of trace quantities of chromium. *J. Lab. Clin. Med. 68*, 484–493.

[7] Gargas, M. L., Norton, R. L., Paustenbach, D. J., and Finley, B. L. (1994) Urinary excretion of chromium by humans following ingestion of chromium picolinate. Implications for biomonitoring. *Drug Metab. Dispos. 22*, 522–529
[8] Offenbacher, E. G., Spencer, H., Dowling, H. J., and Pi-Sunyer, F. X. (1986) Metabolic chromium balances in men. *Am. J. Clin. Nutr. 44*, 77–82.
[9] Anderson, R. A., Colton, T., Doull, J., Marks, J. G., Smith, R. G., Bruce, G. M., Finley, B. L., and Paustenbach, D. J. (1993) Designing a biological monitoring program to assess community exposure to chromium: conclusions of an expert panel. *J. Toxicol. Environ. Health 40*, 555–583.
[10] Anderson, R. A., and Kozlovsky, A. S. (1985) Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr. 41*, 1177–1183
[11] Anderson, R. A. (1987) Chromium in *Trace Element in Human and Animal Nutrition*, 5th Ed. (Mertz, W., ed.) Vol. 1, pp. 225–244, Academic, New York.
[12] Mertz, W., Roginski, E. E., and Reba, R. C. (1965) Biological activity and fate of trace quantities of intravenous chromium (III) in the rat. *Am. J. Physiol. 209*, 489–494.
[13] Fernandez-Lopez, J.-A., Esteve, M., Rafecas, I., Remesar, X., and Alemany, M. (1994) Management of dietary essential metals (iron, copper, zinc, chromium, and manganese) by Wistar and Zucker obese rats fed a self-selected high-energy diet. *BioMetals 7*, 117–129.
[14] Chen, N. S., Tsai, A., and Dyer, I. A. (1973) Effect of chelating agents on chromium absorption in rats. *J. Nutr. 103*, 1182–1186.
[15] Doisy, R. J., Streeten, D. H. P., Freiberg, J. M., and Achneider, A. J. (1976) Chromium metabolism in man and biochemical effects in *Trace Elements in Human and Disease*, *Vol. II, Essential and Toxic Elements* (Prasad, A. S., and Oberleas, D., eds.) pp. 79–104, Academic Press, New York.
[16] Mertz W., and Roginski E. E. (1971) Chromium metabolism: The glucose tolerance factor in *Newer Trace Elements in Nutrition* (Mertz, W., and Cornatzer, W. E., eds.) pp. 123–153, Dekker Press, New York.
[17] Dowling H. J., Offenbacher E. G., and Pi-Sunyer F. X. (1989) Absorption of inorganic, trivalent chromium from the vascularly perfused rat small intestine. *J. Nutr. 119*, 1138–1145.
[18] Seaborn C. D., and Stoecker B. J. (1989) Effects of starch, sucrose, fructose and glucose on chromium absorption and tissue concentrations in obese and lean mice. *J. Nutr. 119*, 1444–1451.
[19] Offenbacher, E. G. (1994) Promotion of chromium absorption by ascorbic acid. *Trace Elem. Electolytes 11*, 178–181.
[20] Urberg, M., and Zemel, M. B. (1987) Evidence for synergism between chromium and nicotinic acid in the control of glucose tolerance in elderly humans. *Metabolism 36*, 896–899.
[21] Hahn, C. J., and Evans, G. W. (1975) Absorption of trace metals in the zinc-deficient rat. *Am. J. Physiol 228*, 1020–1023.
[22] Hill, C. H. (1975) Mineral interrelationships in *Trace Elements in Human Health and Disease* (Prasad, A. S., ed.) pp. 281–300, Vol. 2, Academic, New York.
[23] Hopkins, L. L., Jr, and Schwarz, K. (1964) Chromium(III) binding to serum proteins, specifically siderophilin. *Biochim. Biophys. Acta, 90*, 484–491.
[24] Mertz, W. (1970) Some aspects of nutritional trace element research. *Fed Proc. 29*, 1482–1488.
[25] Davis M. L., Seaborn C. D., and Stoecker B. J. (1995) Effects of over-the-counter drugs on $^{51}$chromium retention and urinary excretion in rats. *Nutr. Res. 15*, 202–210.
[26] Kamath, S. M., Stoecker, B. J., Davis-Whitenack, M. L., Smith, M. M., Adeleye, B. O., and Sangiah, S. (1997) Absorption, retention and urinary excretion of chromium-51 in rats pretreated with indomethacin and dosed with dimethylprostaglandin E2, misoprostol or prostacyclin. *J. Nutr. 127*, 478–482.

[27] Rollinson, C. L., Rosenbloom E., and Lindsay L. (1967) Reactions of chromium(III) with biological substances in *Proceedings of the 7th Intern. Congress on Nutrition*, Vol. 5, pp. 692–698, Pergamon, New York.

[28] Sayato Y., Nakamuro K., Matsui S., and Ando M. (1980) Metabolic fate of chromium compounds. I. Comparative behavior of chromium in rat administered with $Na_2^{51}CrO_4$ and $^{51}CrCl_3$. *J. Pharmacobiodyn. 3*, 17–23.

[29] Brock, J. H. (1985) Transferrins in *Metalloproteins* (Harrison, P. M., ed.) Vol. 2, pp. 183–262, MacMillan, London.

[30] Borguet, F., Cornelis, R., and Lameire, N. (1990) Speciation of chromium in plasma and liver tissue of endstage renal failure patients on continuous ambulatory peritoneal dialysis. *Biol. Trace Elem. Res. 26–27*, 449–460.

[31] Harris D. C. (1977) Different metal-binding properties of the two sites of human transferrin. *Biochemistry 16*, 560–564.

[32] Ani, M., and Moshtaghie, A. A. (1992) The effect of chromium on parameters related to iron metabolism. *Biol. Trace Elem. Res. 32*, 57–64.

[33] Aisen, P., Aasa, R., and Redfield, A. G. (1969) The chromium, manganese, and cobalt complexes of transferrin. *J. Biol. Chem. 244*, 4628–4633.

[34] Tan A. T., and Woodworth R. C. (1969) Ultraviolet difference spectral studies of conalbumin complexes with transition metal ions. *Biochemistry 8*, 3711–3716.

[35] Moshtaghie A. A., Ani M., and Bazrafshan, M. R. (1992) Comparative binding study of aluminum and chromium to human transferrin. Effect of iron. *Biol. Trace Elem. Res. 32*, 39–46.

[36] Ainscough, E. W., Brodie, A. M., Plowman, J. E., Bloor, S. J., Loehr, J. S., and Loehr, T. M. (1980) Studies on human lactoferrin by electron paramagnetic resonance, fluorescence, and resonance Raman spectroscopy. *Biochemistry 19*, 4072–4079.

[37] Ainscough, E. W., Brodie, A. M., and Plowman J. E. (1979) The chromium, manganese, cobalt and copper complexes of human lactoferrin. *Inorg. Chim. Acta. 33*, 149–153.

[38] Sun, Y., Ramirez, J., Woski, S. A., and Vincent J. B. (2000) The binding of trivalent chromium to low-molecular-weight chromium-binding substance (LMWCr) and the transfer of chromium from transferrin and chromium picolinate to LMWCr. *J. Biol. Inorg. Chem. 5*, 129–136.

[39] Sargent, T., III, Lim, T. H., and Jenson, R. L. (1979) Reduced chromium retention in patients with hemochromatosis, a possible basis of hemochromatotic diabetes. *Metabolism 28*, 70–79.

[40] Lim, T. H., Sargent, T., III, and Kusubov, N. (1983) Kinetics of trace element chromium (III) in the human body. *Am. J. Physiol. 244*, R445–R454.

[41] Wilson, J. G., Lindquist, J. H., Grambow, S. C., Crook, E. D., and Maher J. F. (2003) Potential role of increased iron stores in diabetes. *Am. J. Med. Sci. 325*, 332–339.

[42] Yamamoto, A., Wada, O., and Ono, T. (1981) A low-molecular-weight, chromium-binding substance in mammals. *Toxicol. Appl. Pharmacol. 59*, 515–523.

[43] Wada, O., Wu, G. Y., Yamamoto, A., Manabe, S., and Ono T. (1983) Purification and chromium-excretory function of low-molecular-weight, chromium-binding substances from dog liver. *Environ. Res. 32*, 228–239.

[44] Yamamoto, A., Wada, O., and Ono T. (1987) Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. *Eur. J. Biochem. 165*, 627–631.

[45] Yamamoto, A., Wada, O., and Suzuki, H. (1987) Separation of biologically active chromium complex from cow colostrum. *Tohoku J. Exp. Med. 152*, 211–219.

[46] Davis, C. M., and Vincent, J. B. (1997) Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry 36*, 4382–4385.

[47] Sumrall, K. H., and Vincent, J. B. (1997) Is glucose tolerance factor an artifact produced by acid hydrolysis of low-molecular-weight chromium-binding substance? *Polyhedron 16*, 4171–4177.

[48] Vincent, J. B. (2000) Quest for the molecular mechanism of chromium action and its relationship to diabetes. *Nutr. Rev. 58*, 67–72.
[49] Yamamoto, A., Wada, O., and Ono T. (1984) Distribution and chromium-binding capacity of a low-molecular-weight, chromium-binding substance in mice. *Inorg. Biochem. 22*, 91–102.
[50] Kraintz, L., and Talmage, R. V. (1952) Distribution of radioactivity following intravenous administration of trivalent chromium 51 in the rat and rabbit. *Proc. Soc. Exp. Biol. Med. 81*, 490–492.
[51] Visek, W. J., Whitney, I. B., Kuhn, III, U. S. G., and Comar, C. L. (1953) Metabolism of 51Cr by animals as influenced by chemical state. *Proc. Soc. Exp. Biol. Med. 84*, 610–615.
[52] MacKenzie, R. D., Anwar, R. A., Byerrum, R. U., and Hoppert, C. A. (1959) Absorption and distribution of 51Cr in the albino rat. *Arch. Biochem. Biophys. 79*, 200–205.
[53] Vittorio, P. V., Wight, E. W., and Sinnott B. E. (1962) The distribution of chromium-51 in mice after intraperitoneal injection. *Can. J. Biochem. Physiol 40*, 1677–1683.
[54] Hopkins, L. L., Jr. (1965) Distribution in the rat of physiological amounts of injected $Cr^{51}$(III) with time. *Am. J. Physiol. 209*, 731–735.
[55] Onkelinx, C. (1977) Compartment analysis of metabolism of chromium(III) in rats of various ages. *Am. J. Physiol. 232*, E478–E484.
[56] Jain, R., Verch, R. L., Wallach, S., and Peabody, R. A. (1981) Tissue chromium exchange in the rat. *Am. J. Clin. Nutr. 34*, 2199–2204.
[57] Wallach, S., and Verch, R. L. (1989) Correlation between radiochromium distribution and bosy weight in rats. *Trace Elem. Med. 6*, 9–11.
[58] Feng, W. Y., Ding, W. J., Qian, Q. F., and Chai Z. F. (1988) Study on the metabolism of physiological amounts of Cr(III) intragastrical administration in normal rats using activable enriched stable isotope Cr-50 compound as a tracer. *J. Radioanal. Nucl. Chem. 237*, 15–19.
[59] Feng, W. Y., Qian, Q. F., Ding, W. J., and Chai C. F. (2000) Study of chromium speciation in normal and diabetic rats by activable enriched isotope technique. *J. Radioanal. Nucl. Chem. 244*, 321–325.
[60] Feng, W., Bai, L., Jing, L., Zhifang, Ch., Peiqun, Z., Yuxi, G., and Jiujing, Z. (2003) Study of chromium-containing proteins in subcellular fractions of rat liver by enriched stable isotopic tracer technique and gel filtration chromatography. *Anal. Bioanal. Chem. 375*, 363–368.
[61] Vincent, J. B. (1999) Mechanisms of chromium action: Low-molecular-weight chromium-binding substance. *J. Am. Coll. Nutr. 18*, 6–12.
[62] Morris, B. W., Gray, T. A., and MacNeil, S. (1993) Glucose-dependent uptake of chromium in human and rat insulin-sensitive tissues. *Clin. Sci. (Lond) 84*, 477–482.
[63] Morris, B. W., MacNeil, S., Stanley, K., Gray, T. A., and Fraser R. (1993) The interrelationship between insulin and chromium in hyperinsulinaemic euglycaemic clamps in healthy volunteers. *J. Endocrinol. 139*, 339–345.
[64] Clodfelder, B. J., Emamaullee, J., Hepburn, D. D., Chakov, N. E., Nettles, H., and Vincent, J. B. (2001) The trail of chromium(III) from the blood to the urine: The roles of transferrin and chromodulin. *J. Biol. Inorg. Chem. 6*, 608–617.
[65] Clodfelder, B. J., and Vincent, J. B. (2005) The time-dependent transport of chromium in adult rats from the bloodstream to the urine. *J. Biol. Inorg. Chem. 10*, 383–393.
[66] Doisy, R. J., Streeten, D. H. P., Souma, M. L., Kalafer, M. E, Rekant, S. L., and Dalakos, T. G. (1971) Metabolism of $^{51}$Cr in human subjects/normal, elderly and diabetic subjects in *Newer Trace Element in Nutrition* (Mertz, W., and Cornatzer, W. E., eds.) pp. 155–168, Dekker, New York.
[67] Ducros, V. (1992) Chromium metabolism, a literature review. *Biol. Trace Elem. Res. 32*, 65–77.
[68] Anderson, R. A. (1989) Essentiality of chromium in humans. *Sci. Total Environ. 86*, 75–81.
[69] Kozlovsky, A. S., Moser, P. B., Reiser, S., and Anderson, R. A. (1986) Effects of diets high in simple sugars on urinary chromium losses. *Metabolism 35*, 515–518.

[70] Anderson, R. A., Polansky, M. M., Bryden, N. A., Patterson, K. Y., Veillon, C., and Glinsmann W. H. (1983) Effects of chromium supplementation on urinary Cr excretion of human subjects and correlation of Cr excretion with selected clinical parameters. *J. Nutr. 113*, 276–281.

[71] Anderson R. A., Polansky M. M., Bryden N. A., Roginski E. E., Patterson K. Y., Veillon C., and Glinsmann W. (1982) Urinary chromium excretion of human subjects: Effects of chromium supplementation and glucose loading. *Am. J. Clin. Nutr. 36*, 1184–1193.

[72] Donaldson D. L., and Rennert, O. M. (1981) Chromium(III) metabolism by the kidney. *Ann. Clin. Lab. Sci. 11*, 377–385.

[73] Wallach S., and Verch, R. L. (1983) Radiochromium conservation and distribution in diuretic states. *J. Am. Coll. Nutr. 2*, 163–172.

[74] Donaldson, D. L., Smith, C. C., and Yunice, A. A. (1984) Renal excretion of chromium-51 chloride in the dog. *Am. J. Physiol. Renal Physiol. 246*, 870–878.

[75] Hambidge, K. M. (1971) Chromium nutrition in the mother and the growing child in *Newer Trace Element in Nutrition* (Mertz, W., and Cornatzer, W. E, eds.) pp. 169–194, Dekker, New York.

[76] Kandror, K. V. (1999) Insulin regulation of protein traffic in rat adipocyte cells. *J. Biol. Chem. 274*, 25210–25217.

[77] Vincent, J. B. (2000) The biochemistry of chromium. *J. Nutr. 130*, 715–718.

This page intentionally left blank

*Chapter 7*

# Potential and purported roles for chromium in insulin signaling: The search for the holy grail

**John B. Vincent*** **and Randall Bennett**

Department of Chemistry and Coalition for Biomolecular Products, The University of Alabama, Tuscaloosa, AL 35487-0336

## INTRODUCTION

Since the early 1960s, chromium has been associated with insulin (see Chapter 1). The initial proposal for chromium action in insulin signaling involved "glucose tolerance factor," a purported chromium(III) complex of nicotinate, and amino acids. While this work has not stood the test of time, efforts have continued to seek an association between chromium and insulin action. This search has continued despite the questioning of the status of chromium as an essential element; as proposed in Chapter 1, the evidence for an essential role for chromium is best considered suggestive but far from definitive. A separate question regards potential physiological effects from administration of pharmacological, rather than nutritional, quantities of chromium, particularly in subjects with altered glucose metabolism. As discussed in Chapter 5, recent studies strongly suggest that Cr(III) can be used to treat the symptoms of adult-onset diabetes and perhaps other related conditions. These effects appear to be related to an increase in insulin sensitivity. For Cr(III) to have such an effect, presumably Cr(III) or a product of Cr(III) must bind to a biomolecule(s), and this complex must have an altered biological activity compared to that prior to binding chromium. The quest for this chromium biomolecule complex, the "holy grail" of chromium biochemistry, is the focus of this chapter.

## INSULIN SIGNALING

From the appearance of insulin in the bloodstream, the insulin signaling pathway begins with the binding of insulin to the extracellular $\alpha$-subunits of the transmembrane protein insulin receptor (Fig. 1). Insulin receptor consists of two extracellular $\alpha$-subunits and two transmembrane $\beta$-subunits. This binding of insulin turns the receptor into an

* The invited author (and primary author) of this chapter.

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

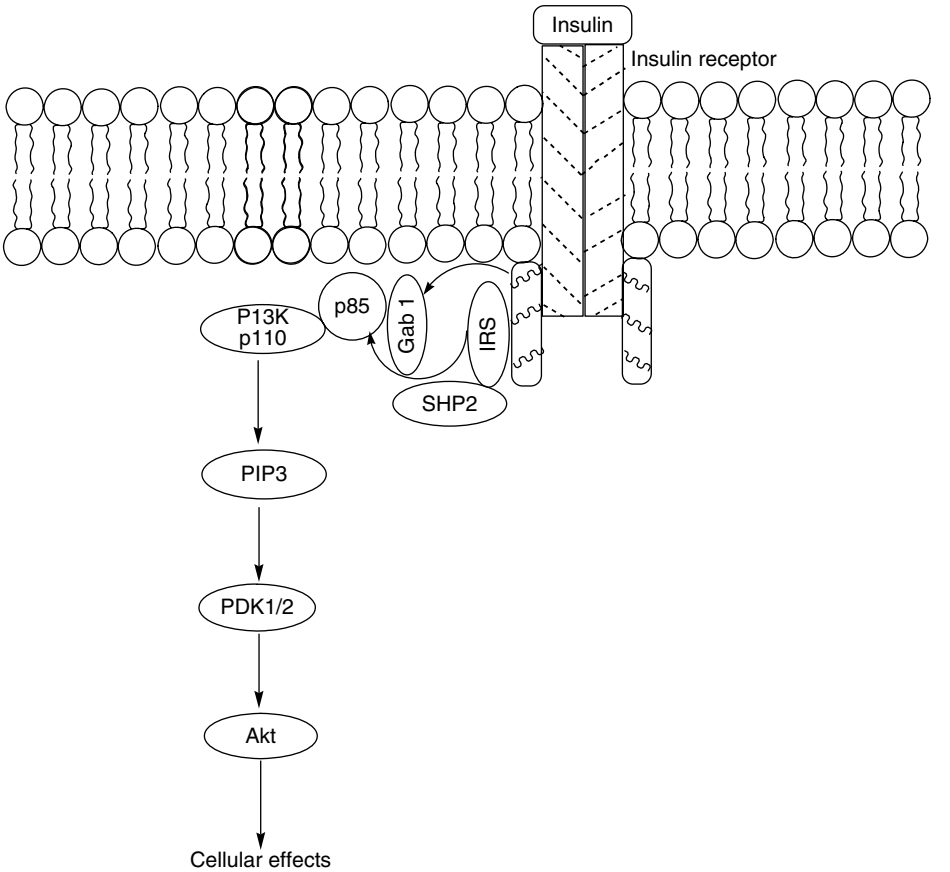



Fig. 1. Insulin signaling pathway.

autokinase, phosphorylating itself at three tyrosine residues (1158, 1162, and 1663 following the human sequence) of the $\beta$-subunit. This conversion turns the receptor into an active kinase catalytically phosphorylating tyrosine residues of several substrate proteins. Known substrates include the insulin receptor substrate proteins (IRS), Shc, Gab-1, and others. These proteins in turn recruit other proteins inside the cell, which possess phosphotyrosine-binding domains (SH2 and PTB domains), forming signaling centers. These adapter molecules include PI3K (phosphatidylinositol 3-kinase) and Grb2. Association of the p85 subunit of PI3K with IRS-1 or Gab activates the catalytic p110 subunit. PI3K in turn phosphorylates serines and threonine residues of Akt (protein kinase B), activating this kinase. Further propagation along this pathway leads to the major cellular effects associated with insulin action, including glucose uptake and metabolism. Thus, enhancing the binding of insulin to its receptor or the activation of any of the kinases along the cascade could enhance insulin signaling.

Alternatively, preventing the signaling system from being deactivated, for example the inhibition of phosphatase enzymes, could also enhance insulin action. Notably,

phosphotyrosine protein phosphatase 1B (PTP1B) has been implicated in the dephosphorylation of insulin receptor. For reviews, see [1, 2].

## LOW-MOLECULAR-WEIGHT CHROMIUM/CHROMODULIN

Two biomolecules are known to bind Cr in vivo: transferrin and low-molecular-weight chromium-binding substance (LMWCr), also termed "chromodulin." Transferrin is responsible for maintaining chromium supplies in the bloodstream and transporting Cr to the tissues [3–6] and possibly from the intestine lining to the bloodstream [7]. The role(s) of LMWCr is less clear.

Low-molecular-weight chromium-binding substance was first reported by the toxicology group of Osamu Wada [8] in 1981. A low-molecular-weight chromium compound was identified by size exclusion chromatography of the cytosol of liver cells of male mice injected with a single dose of potassium chromate. A similar low-molecular-weight compound was found in the feces and urine and 2 hours after injection in the plasma. These researchers suggested that an LMWCr was formed in the liver, which participates in retention and excretion of chromium in the body. The material from the livers of rabbits treated similarly with chromate was partially purified and found apparently to be an anionic organic-chromium complex containing amino acids. This same year, Wu and Wada reported additional studies on LMWCr from urine [9]. Low-molecular-weight chromium-binding substance was found to occur in urine normally, although the amounts were greatly increased after rats were injected with chromate. Normal human and rat urine LMWCr was found not be saturated with chromium. The LMWCr was believed to be similar to that of the liver and other organs of rabbits and dogs and to be involved in removing excess chromium from the body.

Follow-up studies were performed looking at the effects of inhalation exposure of $CrCl_3$. Chromium as LMWCr in the lungs (of rats exposed to the Cr-containing aerosol) slowly decreased while levels in the liver increased. Thus, LMWCr was proposed to be in equilibrium with Cr in the rest of the body; the long half-life of chromium in the lungs was proposed to be the result of low LMWCr levels or a slow rate of synthesis of the LMWCr [10]. Wada and coworkers have also examined the distribution of LMWCr [11]. Low-molecular-weight chromium-binding substance was found in liver, kidney, spleen, intestine, testicle, brain, and blood plasma, with the greatest amount in liver followed by kidney. The organs were obtained from mice 2 hours after injection with potassium dichromate. Supernatants of homogenates of the organs were found to possess more chromium bound to LMWCr when dichromate was added to the homogenate than when the mice were injected with dichromate. The time course of chromium binding to LMWCr after injection of dichromate was also examined [11]. Chromium was found to be associated with liver and kidney LMWCr only 2 minutes after injection and reached a maximum within 1–2 hours after treatment. In these studies, LMWCr was again identified by its elution behavior in size exclusion chromatography, and its Cr-binding ability.

Efforts have continued to isolate and characterize LMWCr. To date LMWCr has been isolated and purified from rabbit liver [12], bovine liver [13], porcine kidney [14], and porcine kidney powder [14] and partially purified from dog [15] and mouse liver [11]. Inclusion of protease inhibitors in buffers during the isolation of bovine

liver LMWCr does not affect the amount of oligopeptide isolated [13], suggesting it is not a proteolytic artifact generated during the isolation procedure. The materials from rabbit and dog liver were loaded with Cr by injection of the animal with chromate (or Cr(III) which provides lower yields). For the materials from bovine liver and porcine kidney and kidney powder, chromate was added to the homogenized liver or kidney or suspended kidney powder. Chromium(III) could also be added to the bovine liver homogenate to load LMWCr with Cr, but the loading was not as efficient as when chromate was utilized [13]. A Cr-loading procedure is required so that the material can be followed by its Cr content during the isolation and purification procedures [12, 13]. The isolation procedures are similar involving an ethanol precipitation, anion exchange chromatography, and finally size exclusion chromatography. Thus, LMWCr appears to be a naturally occurring oligopeptide composed of glycine, cysteine, aspartate, and glutamate with the carboxylates comprising more than half of the total amino acid residues (Table 1). The amino acid composition data for the rabbit liver LMWCr (injected chromate) and bovine liver (chromate added to homogenate) are extremely similar, indicating that the type of Cr-loading procedure utilized is probably not critical to the composition of the isolated material. No amino acid sequence data has appeared, despite attempts at sequencing by Edman degradation, nuclear magnetic resonance (NMR), and mass spectrometry. The lack of additional characterization of the organic components of the materials is a matter of concern. Additionally, note that the material from urine has not been isolated and characterized; its assumed identity with the material from liver is based solely on their similar apparent molecular weight from size exclusion and similar chromatographies and their chromium-binding potential. Unfortunately to date, LMWCr has not proven to be antigenic, preventing its presence to be detected using immunological techniques.

The amino acid composition of LMWCr has been used to search the human genome for protein possessing fragments comprised of these amino acids. Two candidate sequences, EDGEECDCGE and DGEECDCGEE, from the beginning of the disintregrin domain of the protein ADAM 19 have been identified [17, 18]. ADAM 19 is a multidomain membrane protein with disintegrin and metalloproteinase domains [19]. The ten-amino acid sequences are conserved in rat, mouse, and human ADAM 19, the only

Table 1
Amino acid composition data for isolated LMWCr's

| Source | Glycine | Glutamic acid | Aspartic acid | Cysteine | Ref. |
|---|---|---|---|---|---|
| Rabbit liver | 3.22 | 3.91 | 1.98 | 1.75 | 12 |
| Dog liver | 2.71 | 4.3 | 1.00* | 2.23 | 15 |
| Bovine liver | 2.47 | 4.47 | 2.15 | 2.19 | 13 |
| Bovine colostrum | 1.98 | 5.0 | 4.12 | 0.93 | 16 |
| Porcine kidney | 1.45 | 4.05 | 2.31 | 0.622 | 14 |

* For the dog liver material (only partially purified), ratios are based on the assumption of only one aspartic acid residues per oligopeptide; amino acid analysis indicated the presence of significant amounts of threonine, serine, and leucine.

sources for which the sequence is known. The first sequence is also present in a putative ADAM in fission yeast [17]. The location of these sequences is at the very end of an exon, suggesting the possibility that LMWCr could derive from an alternative splicing product. This is an area requiring more extensive investigation, and only preliminary results of chromium binding to a peptide with the former sequence have been reported [18].

Despite its small size (approximately 1500 molecular weight; 1438 by MALDI-TOF mass spectrometry for bovine liver LMWCr) [5], the molecule from rabbit and bovine liver tightly binds four equivalents of chromic ions. The binding is quite tight ($K_a \sim 10^{21}$ $M^{-4}$ for bovine liver) and highly cooperative (Hill coefficient, $n = 3.47$) [20]; thus, essentially only apoLMWCr and holoLMWCr ($Cr_4$-LMWCr) co-exist in solution. ApoLMWCr can accept chromic ions from biological molecules including $Cr_2$-transferrin [11, 20]. Spectroscopic studies suggest that the chromic ions comprise an anion-bridged multinuclear assembly supported by carboxylates from the oligopeptide [13, 21]. Electronic studies clearly indicate that the Cr bound to LMWCr exists in the trivalent oxidation state; for the bovine liver material, 10Dq and the Racah parameter B were found to be $1.74 \times 10^3$ and 847 $cm^1$, respectively [13], indication of predominately oxygen-based coordination. In the ultraviolet region, the spectra of LMWCr's possess a maximum or shoulder at approximately 260 nm; this feature may arise from a disulfide linkage [22]. Paramagentic $^1$HNMR spectroscopy of the bovine liver reveals a downfield-shifted resonance at approximately +45 ppm, suggestive of the protons of a methylene carbon bound adjacent to a carboxylate bridging two chromic centers [13]. The presence of a bridging ligand suggests the existence of a multinuclear assembly. X-ray absorption spectroscopic studies on the bovine liver LMWCr have shown that the chromium atoms are surrounded by six oxygen atoms at an average distance of 1.98 Å and are consistent with a lack of sulfur-based ligands [21]. A long Cr . . . Cr interaction at ~3.79 Å is present, and another such interaction may be present at 2.79 Å. This is also consistent with the presence of a multinuclear Cr assembly. As holoLMWCr can be prepared simply by addition of chromic ions to solutions of apoLMWCr, anionic bridges to the chromium assembly are probably hydroxide ions [13, 23]; the X-ray absorption studies failed to detect any short Cr-oxo interactions [21]. The nature of the assembly has been narrowed to a few possibilities from electron paramagnetic resonance (EPR) spectroscopy and variable temperature magnetic susceptibility measurements [21]. X-band EPR studies indicate that at least three chromic ions are coupled to give a species with an $S = 1/2$ ground state giving rise to a broad signal at g~2; this signal appears to be broadened by interaction with another Cr species giving rise to a complex EPR signal centered about g~5. The g~2 EPR signal sharpens as the temperature is raised from 5 to 30 K, suggesting that dipolar coupling exists between the two Cr species giving rise to the EPR signal. Finally, magnetic susceptibility studies are consistent with the presence of a mononuclear chromic center and an unsymmetric trinuclear chromic assembly [21]. Put together, the spectroscopic and magnetic data on bovine LMWCr suggest the occurrence of a $Cr_4$ assembly in LMWCr. The Cr environment is mostly, if not exclusively, composed of O atoms, and the assembly is comprised of a single chromic ion and a trinuclear unit. Additionally, the sulfur atoms of the two cysteine residues of LMWCr appear to be involved in a disulfide linkage and not in binding chromium. Similarly, the N-terminal amine group can be derivatized, suggested it is not coordinated to chromium. Thus, oligopeptide-provided ligands appear to be limited to

carboxylates from the side chains of the aspartate and glutamate residues and possibly the carboxy terminus. One laboratory has reported not being able to isolate bovine liver LMWCr while instead isolating a material they identified as containing Cr(IV) or Cr(V) [24]. Much of the work in this chapter has been refuted [25], and the existence of such a Cr(IV) or Cr(V) species with the stability necessary to be isolated under these conditions is difficult to fathom.

A related chromium-containing oligopeptide from bovine colostrum (M-LMWCr) is comprised of the same amino acids but in distinctly different ratios and also stimulates insulin-dependent glucose metabolism in rat adipocytes [16, 26]. Whether the oligopeptide is present in other forms of milk is unknown. The significance of these differences between the liver and colostrum oligopeptides is essentially unexplored. The existence of multiple forms of LMWCr in cows raises concerns about identifying LMWCr in tissues or body fluids from only its apparent molecular weight and chromium-binding ability. For example, are blood and urine LMWCr fractions comprised of one or more of these oligopeptides?

Curiously, the oligopeptide is maintained in the soluble portion [11] and the nucleus [5] of liver cells in the apo form. As noted above, the oligopeptide is isolated as the holo-oligopeptide (so that it may be followed in the purification schemes by its chromium content), which means that an in vivo or in vitro chromium-loading step is required [12, 13]. This observation has resulted in the suggestion that LMWCr may play a role in chromium detoxification; however, injection of chromic ions or chromate into mice does not stimulate the production of LMWCr [11]. Thus, while LMWCr does carry chromium into the urine after intake of large dosages of Cr(III) or Cr(VI) [15], the suggested detoxification role of LMWCr is unlikely to be its primary function.

Insulin dose response studies using rat adipocytes have indicated a potential intrinsic biological function for LMWCr. Isolated rat adipocytes in the presence of LMWCr and insulin display an increased ability to metabolize glucose to produce carbon dioxide or total lipids; this increase occurs without a change in the insulin concentration required for half maximal stimulation [16, 26, 27]. This lack of change in half-maximal insulin concentration suggests a role for LMWCr inside the insulin-sensitive cells after insulin binds externally to the insulin receptor [27]. The stimulation of glucose metabolism by LMWCr is proportional to the chromium content of the oligopeptide [28].

While insulin dose response studies with LMWCr suggested a role inside the cells and because the primary events between insulin binding to its receptor and glucose transport are signal transduction events, that is phosphorylation/dephosphorylation of protein residues, a role for LMWCr in these events has been probed. LMWCr has been shown to activate the tyrosine kinase activity of insulin-activated insulin receptor [23] and to activate a membrane phosphotyrosine phosphatase in adipocyte membranes [29]. For example, the addition of bovine liver LMWCr to rat adipocytic membranes in the presence of 100 nM insulin results in a concentration dependent up to eightfold stimulation of insulin-dependent protein tyrosine kinase activity, while no activation of kinase activity is observed in the absence of insulin [28]. The dependence of the kinase activation on the concentration of LMWCr can be fit to a hyperbolic curve to give dissociation constants ($K_m$'s) of approximately 875 pM, indicating extremely tight binding. Blocking the insulin-binding site on the external $\alpha$-subunit with antibodies, whose epitope lies in this region, results in the loss of the ability to activate insulin

receptor kinase activity [28]. Examining the potential activation of isolated rat insulin receptor by bovine liver LMWCr in the presence of insulin indicates that LMWCr can amplify the isolated receptor protein tyrosine kinase activity by approximately sevenfold, conclusively demonstrating that the receptor is the site of interaction with LMWCr. Fitting the activation curve to a hyperbolic function gives a dissociation constant of approximately 250 pM [28]. The site of LMWCr binding on insulin receptor can be further refined. Studies with a catalytically active fragment (residues 941–1343) of the $\beta$-subunit of human insulin receptor (which does not require insulin for kinase activity) reveal that LMWCr can stimulate kinase activity threefold with a dissociation constant of 133 pM. Thus, LMWCr binds at or near the kinase active site [30].

Chromium plays a crucial role in the in vitro activation of insulin receptor kinase activity by LMWCr [28]. ApoLMWCr displays little ability to activate insulin-dependent tyrosine kinase activity in the rat adipocyte membranes, with the small amount of activity readily attributable to residual chromium. However, titration of apoLMWCr with chromic ions results in the total restoration of the ability to activate kinase activity; approximately, four chromic ions per oligopeptide are required for maximal activity. This is consistent with the number of chromium (four per oligopeptide) reported to be bound to holoLMWCr from liver sources. The activity of LMWCr is rapidly restored upon the addition of chromium, consistent with binding studies which indicate that Cr binding is highly cooperative, such that holoLMWCr forms rapidly to the exclusion of complexes of LMWCr without its full complement of chromium. This reconstitution of LMWCr's activation potential is specific to chromium. Transition metal ions other than chromium which are commonly associated with biological systems (V, Mn, Fe, Co, Ni, Cu, Zn, and Mo) are ineffective in potentiating the ability of apoLMWCr to activate kinase activity. In fact, all the ions except $Cr^{3+}$ resulted in loss of activation potential relative to apoLMWCr. Similarly, the metal ions themselves (in the absence of apoLMWCr) are ineffective in activating the insulin-dependent kinase activity. Thus, the ability of LMWCr to potentiate the effects of insulin in stimulating the insulin-dependent protein tyrosine kinase activity of insulin receptor is specific to chromium and is directly dependent on the chromium content of LMWCr.

The activation of a membrane-associated phosphotyrosine protein phosphatase (PTP) by LMWCr has been little explored. Studies with isolated LAR and PTP1B have shown that chromodulin has no effect on these phosphatases [29, 31]. One group [32] has reported that the results of references [29, 31] on human PTP's are contradictory; however, both studies are in agreement in that no activation of isolated PTP's was observed [29, 31].

Based on these results and additional studies (vide infra), LMWCr has been proposed to function as part of a unique autoamplification system for insulin signaling (Fig. 2) and a new (and shorter) name, chromodulin, has been put forward [33, 34]. In this mechanism, apoLMWCr is stored in insulin-sensitive cells. In response to increases in blood insulin concentrations (as would result from increasing blood sugar concentrations after a meal), insulin binds to its receptor bringing about a conformation change which results in the autophosphorylation of tyrosine residues on the internal side of the receptor. This transforms the receptor into an active tyrosine kinase and transmits the signal from insulin into the cell. In response to insulin, chromium is moved from the blood to insulin-sensitive cells. Here, the chromium flux results in the loading of apoLMWCr

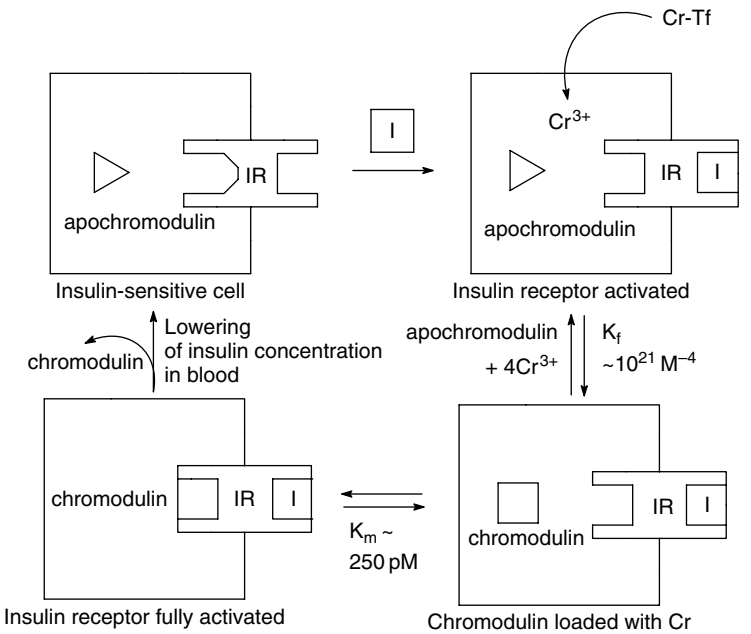



Fig. 2. Proposed mechanism for the activation of insulin receptor kinase activity by chromodulin in response to insulin. The inactive form of the insulin receptor (IR) is converted to the active form by binding insulin (I). This triggers a movement of chromodulin (presumably in the form of chromium transferrin, Cr-Tf) from the blood into insulin dependent cells, which in turn results in a binding of chromium to apochromodulin (triangle). Finally, the holochromodulin (square) binds to the insulin receptor, further activating the receptor kinase activity. Apochromodulin is unable to bind to the insulin receptor and activate kinase activity. When the insulin concentration drops, holochromodulin is released from the cell to relieve its effects. Reproduced from Ref. [43] with the permission of the copyright holder.

with chromium. The holoLMWCr then binds to the receptor, presumably assisting to maintain the receptor in its active conformation, amplifying its kinase activity. When the signaling is to be turned off, a drop in blood-insulin levels facilitates relaxation of the conformation of the receptor, and the holoLMWCr is excreted from the cell into the blood. Ultimately, LMWCr is efficiently excreted in the urine. The basis of the alternative name chromodulin is the similarity of the proposed mechanism of action to that of the calcium-binding protein calmodulin [35]. Both bind four equivalents of metal ions in response to a metal ion flux; however, the four calcium ions which bind to the larger protein calmodulin rest in mononuclear sites. Both holoproteins selectively bind to kinases and phosphatases stimulating their activity.

The model is also based on human studies. In human euglycemic hyperinsulinemic clamp studies, Morris and coworkers have shown that increases in blood insulin concentrations following an oral glucose load result in significant decreases in plasma chromium levels; a subsequent infusion of insulin led to further chromium losses [36]. Within one–and-a-half hours after the increases of blood insulin concentrations, blood chromium levels started to recover. Patients also showed increased urinary chromium

losses during the course of the experiments, with the amount of chromium lost roughly corresponding to the amount of chromium estimated to be lost from the intravascular space [36]. Numerous other studies have demonstrated that chromium is released in urine within 90 minutes of a dietary stress, for example due to high sugar intake [37–42]. As glucose tolerance as a result of repeated application of carbohydrate stress decreases, the mobilization of chromium and resulting chromium loss have been shown to decrease [39]. These homeostasis and urinary output studies suggest that chromium stored or maintained in the blood is mobilized in response to increases in blood insulin concentrations where ultimately it appears in the urine.

Recently, a proposal for chromium transport from the bloodstream to the urine has been presented (Fig. 3) [43]. Transferrin has been known for decades to bind injected or orally administered Cr(III) (>80% of plasma Cr is bound to transferrin) [3]. Recently, transferrin has been shown to be responsible for the transport of Cr from the blood stream to the tissues. Most recently, injection of $^{51}$Cr-labeled transferrin into the bloodstream has been shown to result in a rapid and insulin-sensitive movement of chromium into the tissues as Cr-transferrin; greater than 50% of the Cr is transported to the tissues within 30 minutes [6]. Tissue levels of Cr are maximal 30 minutes after injection; decreases in tissue Cr with time are mirrored by increases in urine Cr. Approximately 50% of the $^{51}$Cr appears in the urine with 360 minutes of injection in the absence of added insulin; insulin treatment concurrent with injection of $^{51}$Cr-labeled transferrin results in ~80% of the label appearing in the urine within 180 minutes. The removal of $^{51}$Cr from the blood is faster than the appearance of $^{51}$Cr in the urine; the lag in time indicates that the Cr-transferrin in the blood and Cr in the urine are not in direct equilibrium and that intermediates in the transport of Cr must be involved. This establishes a clear pathway of transport of Cr starting from transport by transferrin from the bloodstream into the tissues, followed by release and processing in the tissues to form LMWCr, excretion into the bloodstream, rapid clearance of LMWCr or a similar species into the urine, and ultimately excretion as this species. Insulin stimulates the processing of Cr in the tissues [6]. While this data on chromium transport has been incorporated into the proposed mechanism of enhancement of insulin signaling by LMWCr, it will need to be incorporated into any complete mechanism of in vivo chromium action.

It is important to note that all the studies on LMWCr and insulin receptor are in vitro. This proposed mechanism of action of chromium needs to be supported by in vivo studies. Does LMWCr bind to insulin receptor in vivo and is insulin receptor kinase activity affected in vivo by interacting directly with LMWCr in vivo? The only reported in vivo studies with LMWCr are of a quite different nature. $LD_{50}$ [11] and mean tubular reabsorption rates [15] have been measured as noted above. Additionally, intravenous treatment of rats with 20 μg of chromium as LMWCr per day for 12 weeks has been shown to have little, if any, effect on rats [44]. Thus, in terms of its use as a dietary supplement or therapeutic compound, LMWCr appears to be recognized and readily excreted. Injection of LMWCr into rabbits has been shown to lead to rapid excretion of chromium, especially compared to the use of other forms of chromium; this is reflected in the mean tubular reabsorption rate for LMWCr of 23.5% in contrast to rates of 85.7 and 92.5% for chromate and chromium chloride, respectively [15]. This is probably also responsible for the extremely high $LD_{50}$ for LMWCr injected into mice of 135 mg/kg

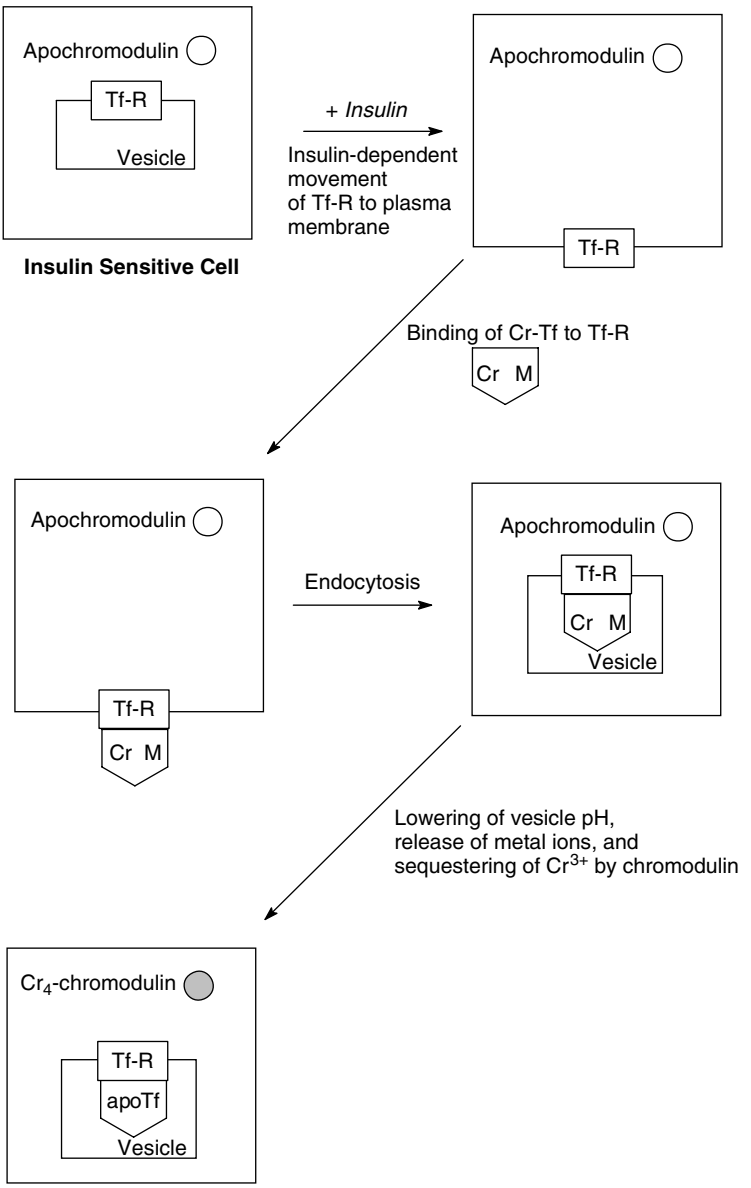



Fig. 3. Proposed mechanism for the movement of chromium from blood to chromodulin. In response to increases in plasma insulin concentrations, transferrin receptor (Tf-R) in insulin-sensitive cells migrates from vesicles to the plasma membrane. Transferrin (pentagon), which contains two bound metal ions (in this case one chromic ion and one other metal cation (M)), binds to the receptor and is internalized by endocytosis. The pH of the resulting vesicle is reduced by ATP-driven proton pumps, resulting in release of the metal ions from transferrin. Chromium released from multiple transferrin molecules is sequestered by apochromodulin (open circle) to produce chromium-loaded chromodulin (dark circle). Reproduced from Ref. [43] with the permission of the copyright holder.

body mass [11]. These studies looking at effects of injection LMWCr need to followed with studies designed to probe the fate and potential action of holoLMWCr generated in the tissues.

**Synthetic models**

The recent research on LMWCr has inspired synthetic efforts to prepare new chromium–carboxylate assemblies. Known assemblies with nuclearity greater than two (but less than eight) possess four types of cores: symmetric [45] and unsymmetric [46] $Cr_3O$, $Cr_3(OH)_2$ [47], and $Cr_4O_2$ [48–50]. Numerous examples of the type containing the symmetric $Cr_3O$ core have been well-characterized, and interest in these complexes date back to the late nineteenth century [45]. The other cores have been prepared only during 1990s using the symmetric trinuclear complexes as starting materials.

Given the novel role in the autoamplification of insulin signal transduction for LMWCr and its rather simple composition (carboxylate-rich oligopeptide binding four chromic ions), attempts have been made to identify a functional model for chromodulin. Such a biomimetic would be required to be soluble and stable in aqueous solution. Few of the known trinuclear and tetranuclear Cr(III) oxo(hydroxo)-bridged carboxylate assemblies are soluble in water. On the basis of these requirements, two assemblies have been examined: $[Cr_3O(O_2CCH_3)_6(H_2O)_3]^+$ and $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ [30]. Both possess the symmetric basic carboxylate-type structure comprising of a planar triangle of chromic ions with a central $\mu_3$-oxide. The acetate complex does not activate the tyrosine protein kinase activity of the active site fragment of insulin receptor or of adipocytic membrane fragments in the presence of insulin and actually inhibits the activity. In stark contrast, the propionate analog activates the kinase activities in a fashion very similar to LMWCr. The kinase activity of the isolated receptor fragment, for example, is stimulated approximately threefold with a dissociation constant of 1.00 nM [30]. (One laboratory has reported not detecting stimulation of insulin receptor kinase activity by this complex.) [32]. The complex appears to be a functional biomimetic for LMWCr, and this was previously used as support for the existence of a multinuclear chromic assembly in LMWCr. However, the recent spectroscopic and magnetic studies on LMWCr clearly indicate that LMWCr does not possess a $Cr_{3-}\mu_3\text{-}O^{2-}$ core as does the synthetic compound. Additionally, LMWCr is anionic while the synthetic species is cationic. Thus, while the two species possess anion-bridged multinuclear Cr(III) assemblies bridged by carboxylate ligands, they possess distinctly different structures and charges such that any functional similarities must be considered coincidental.

The propionate biomimetic has been found to have a striking in vivo effect, lowering plasma triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol levels after 12 weeks of supplementation in rats at a rate of 20 μg Cr/kg body mass daily and potentially lowering body mass and fat content [44]. The in vivo effects of administration of the synthetic cation $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ on healthy and type 1 and type 2 diabetic model rats have been examined. (The synthetic cation was initially given intravenously to avoid potential differences in absorption between the healthy and the diabetic model rats). After 24 weeks of intravenous administration (0–20 μg Cr/kg body mass) to healthy male rats, the cation resulted in a concentration-dependent lowering of levels of fasting blood plasma LDL cholesterol, total cholesterol, triglycerides, and insulin and of 2-hour plasma insulin and glucose levels after a glucose

challenge. These results confirmed the result of previous 12-week study examining the effect of the synthetic cation on healthy rats [44] and are in stark contrast to those from administration of other forms of Cr(III) to healthy rats, which have no effect on these parameters. The cation had little, if any, effect on rats with streptozotocin-induced (type 1 model) diabetes. This may result from the increased spread of the values of the blood variables in these rats compared to those of controls, such that insufficient power existed to observe any potential effects. However, Zucker obese rats (a early stage type 2 diabetes model) after 24 weeks of supplementation (20 μg/kg) have lower fasting plasma total cholesterol, triglyceride, insulin, and LDL and HDL cholesterol levels and lower 2-hour plasma insulin levels after a glucose challenge. The lowering of plasma insulin concentrations with little effect on glucose concentrations suggests that the supplement increases insulin sensitivity. No acute toxic effects were observed for supplementation with the compound, and it does not give rise to DNA damage in in vitro studies as observed with chromium picolinate [51].

The effects of oral (gavage) administration of the complex have been examined [52]. At levels of 250, 500, or 1000 μg Cr/kg body mass, the treatment at all concentrations lowered fasting plasma insulin, triglycerides, total cholesterol, and LDL cholesterol levels of healthy rats while having no effect on plasma glucose or HDL cholesterol. These levels were lower after 4 weeks of treatment and remained lower for the next 20 weeks of treatment. The maintenance of glucose levels with less insulin indicates increased insulin sensitivity. Both plasma glucose and insulin levels were lowered in 2-hour glucose tolerance tests. Previously, Anderson and coworkers have shown that $CrCl_3$ and chromium picolinate when given orally up to 100 mg Cr/kg food (equivalent to ~8 mg Cr/kg body mass, eight times the largest amount administered in this study and, after correcting for absorption differences, approximately equivalent to the highest dose in this study) had no effect on any of these plasma variables [52]. Effects on healthy rats appear to be unique to the biomimetic complex. Additionally, the healthy rats receiving the largest dose of Cr tended to possess less body mass than controls and had ~10% less epididymal fat; this tendency toward less body mass (also observed in earlier study with the biomimetic) and loss of fat (if confirmed by additional studies) also point toward unique effects from the biomimetic. In Zucker obese rats, the early stage type 2 diabetes model, receiving 1000 μg Cr/kg body mass, the results were similar to those from intravenous administration. In this study, the effects of the biomimetic cation on Zucker Diabetic Fatty (ZDF) rats, a genetic model for type 2 diabetes, were also examined using 1000 μg Cr/kg body mass. Again fasting plasma insulin, triglycerides, total cholesterol and LDL cholesterol levels were all lower while glucose concentrations were consistently but not statistically lower. HDL levels were lowered from their very high levels. Two-hour plasma insulin levels were also lowered. Plasma-glycated hemoglobin levels, a measure of longer-term blood glucose status, were examined in the healthy, Zucker obese, and ZDF rats after 4, 12, and 24 weeks of treatment. No effect was seen for the healthy rats; however, significant effects were noted for the diabetic models. For the ZDF rats, glycated hemoglobin was lower after 12 and 24 weeks of treatment, reaching almost a 22% drop compared to ZDF controls by week 24; for the Zucker obese rats, glycated hemoglobin was 27% lower at week 24. Control studies using an intravenous injection containing an amount of propionate equivalent to that received in the largest dose used above have not observed similar effects [53].

The effects of the cation on healthy and model diabetic rats have also been examined by Debski and coworkers. Male Wistar rats were provided a control diet or a diet containing 5 mg Cr/kg as the cation for 10 weeks [54]. Blood plasma insulin levels were lowered 15.6% by the Cr-containing diet, while glucose transport by red blood cells was increased 9.6%. In another study, this group utilized male Wistar rats with streptozotocin-induced diabetes. Using similar diets for 5 weeks, the rats had the Cr diet with lower blood serum glucose levels (26%) and increased HDL levels (14%) [55].

During the first 24 hours after intravenous injection, the fate of the $^{51}$Cr-labeled complex in tissues, blood, urine, and feces has been followed [56]. Remarkably, the complex is readily incorporated into tissues and cells. The complex rapidly disappears from the blood (<30 minutes) as radio-labeled Cr from the cation appears in tissues. In hepatocytes, the intact cation is efficiently transported into microsomes where its concentration reaches a maximum in approximately 2 hour (and corresponds to >90% of Cr in the cells from the injected complex); this suggests that the cation is actively transported into cells via endocytosis; identification of the protein(s) responsible is needed. As the complex is degraded in hepatocytes and the levels in microsomes rapidly decrease, Cr appears in the urine as chromodulin (or a similar molecular-weight chromium-binding species (vide supra)). The synthetic complex is degraded before or during its disappearance from the microsomes. During the initial periods when the blood concentration of the complex is high, some of the complex passes into the urine intact [56]. The rats have also been given the $^{51}$Cr-or $^{14}$C-labeled complex by intravenous injection daily for 2 weeks [57]. Thirty per cent of the injected Cr is lost daily as chromodulin or a similar species; only very small amounts are lost in the feces. The tissue and subcellular hepatocyte distribution of chromium after 2 weeks was examined; no intact complex could be detected. Degradation of the complex resulted in accumulation of Cr in the liver and kidney as observed with other Cr sources. Only ~3% of the propionate from each injection of the complex was lost daily; the tissue and cellular distribution of derivatized propionate after 2 weeks varied greatly from that of Cr. Thus, the active transport of the biomimetic is different from the transport of all other synthetic forms of Cr proposed as dietary supplements; these proposed supplements appear to enter cells passively by diffusion. Hence, the biomimetic complex has a significantly greater ability to enter cells than other Cr supplements and can bring about positive changes in carbohydrate and lipid metabolism unlike other chromium supplements.

At a nutritionally relevant level (3 μg Cr/kg body mass) and a pharmacologically relevant level (3 mg/kg), at least 60 and 40% of the compound, respectively, is absorbed in 24 hours (Fig. 4) [58]. This represents approximately a tenfold increase over those of chromium picolinate (marginally soluble in water, 0.6 mM), $CrCl_3$ (which oligimerizes in water), and chromium nicotinate ("$Cr(nic)_2(OH)$," insoluble in water). The solubility of the biomimetic cation and its stability, thus, allow a unique amount of the material to enter the circulatory system and tissues.

## DIRECT CHROMIUM BINDING TO INSULIN RECEPTOR

Another proposal for chromium-activating insulin receptor related to the LMWCr mechanism has been proposed by Van Horne and coworkers [17]. Noting that the amino acid

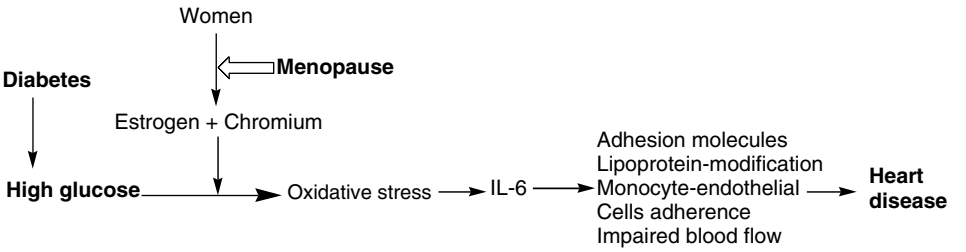



Fig. 4. Proposed mechanism of how estrogen and chromium could beneficially affect heart disease. Based on Ref. [78].

composition of LMWCr was approximately 4 Glu: 2 Asp: 2 Cys: 2 Gly or for the smallest whole number ratio 2 Glu: 1 Asp: 1 Cys: 1 Gly, these workers postulated that LMWCr could be comprised of two linked-pentameric sequences and searched the sequences of a set of proteins related to insulin signaling for segments with the composition 2 Glu: 1 Asp: 1 Cys: 1 Gly. They identified an acidic region on the $\alpha$-subunit of insulin receptor with the sequence EECGD (residues 175–184). They proposed a structure for LMWCr where the four metal centers are bound to two equivalent of the pentamer, which are held together by a disulfide linkage. A synthetic peptide derived from this sequence was shown from a Cr(III) complex with a proposed formula of $Cr_3O$ [17]. The basis for the formula is not presented.

Based on these results, Van Horn and coworkers have proposed multinuclear chromium assemblies that bind to the $\alpha$-subunit of insulin receptor in conjunction with insulin binding activating the receptor's kinase activity. The binding of chromium would be to these pentameric sequences in the $\alpha$-subunit. After insulin is degraded, the chromium assembly or Cr-assembly-peptide complex is released by a mechanism which is yet to be determined to return the receptor to its inactive form. This extracellular Cr(III) cycling mechanism must be considered highly speculative at present.

## AKT

Cefalu [59] in a preliminary report has indicated that chromium picolinate may act by increasing the activation of Akt phosphorylation. In this study, eight type 2 diabetic subjects received 1 mg Cr in the form of chromium picolinate while eight subjects received placebos; the diabetic subjects receiving Cr after insulin treatment had increased Akt phosphorylation but not IR or IRS-1 phosphorylation. Increased Akt phosphorylation was suggested to be the mechanism of chromium action. Yet, at a CADRE Research summit in 2003, Cefalu [60] reported results of a similar study using JCR: LA-corpulent rats, a model of insulin-resistant cardiovascular disease. (Cefalu had previously reported that daily oral administration of chromium picolinate (18 μg Cr/kg body mass) to the rats for 12 weeks resulted in lower plasma total cholesterol and high HDL cholesterol and lower fasting insulin levels.) [61]. No effects were observed with healthy controls. Chromium picolinate treatment resulted in increased IR, IRS-1, and Akt phosphorylation and increased PI-3 kinase activity after insulin administration. These results are actually

consistent with Cr acting at insulin-stimulated insulin receptor, leading to the other effects downstream in the signal cascade.

Sreejayan and coworkers [62] using a different Cr complex have generated additional evidence for an association between Cr and Akt. $Cr(phenylalanine)_3$ (5 or 25 μM for 10 days) was found to increase insulin-stimulated glucose uptake by cultured mouse 3T3-adipocytes. Treatment of the cells with 5 μM Cr for 0.5–4 hours or 0.1 to 100 μM Cr for 2 hours did not increase insulin-stimulated phosphorylation of insulin receptor (Tyr 1146) significantly, while under similar conditions insulin-stimulated Akt phosphorylation (Thr 308) was increased significantly.

## CHROMATE

Lay and coworkers [32, 63] have proposed that chromate generated enzymatically (i.e., from hydrogen peroxide or other species generated by enzymes) from Cr(III) in the body could act as a phosphotyrosine phosphatase (PTP) inhibitor, in a similar manner to vanadate, and that the site of action of Cr is at PTP's. Chromate has been shown in cell studies to have insulin-mimetic effects similar to vanadate [64–67]. However, several studies have shown that streptozotocin-treated rats (which are insulin deficient) do not show benefit from chromium administration [44, 68, 69], while numerous studies have shown that vanadium can serve as an insulin substitute in these rats [68, 69]. Despite the nature of the vanadium compound utilized, the active species in the inhibition appears to be "naked" vanadate [70]. Vanadate increases basal levels of phosphorylation of IR and IRS-1 and activity of PI3-kinase, but these levels are not further stimulated by insulin treatment [71]. Additionally, the increased phosphorylation of Akt and IR in response to vanadate displays different vanadate concentration dependence [72], as might be expected if different PTP's are involved in inactivation. Consequently, if chromate is responsible for the in vivo effects of chromium then examining the effects of Cr on the insulin cascade system in vivo both before and during insulin stimulation should be distinctly different (i.e., vanadate-like) than that if Cr acts by stimulating insulin receptor kinase activity.

The proposal that chromate could be involved in chromium action in vivo is based on the ability of hydrogen peroxide to oxidize Cr(III) compounds to chromate [32]. To demonstrate this, Lay and coworkers exposed chromium picolinate, $CrCl_3$, and the basic chromium carboxylate cation $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ to 0.10–1 mM hydrogen peroxide for 1–6 hours in 0.10 M HEPES buffer, pH 7.4. This resulted in the formation of chromate in efficiencies from 1 (chromium picolinate for 6 h with 1 mM $H_2O_2$) to 33% (the cation for 6 hours with 1 mM $H_2O_2$). The cation could also be oxidized with hypochloride, or glucose oxidase, or xanthine oxidase (enzymes that produce $H_2O_2$).

To put the potential for chromate to be the biological active form of chromium in perspective, some simple calculations are in order. Over-the-counter chromium supplements and chromium supplements used in human studies generally provide 200 μg–1 mg of Cr daily. Using the best-selling and most studied supplement chromium picolinate as an example, absorption is estimated to be about 2% [73, 74]. If one assumes 1 mg dose with 5% absorption, a human would have 50 μg Cr enter the bloodstream. Using the atomic mass of Cr of 52 g/mol, this represents approximately 1 μmole Cr. Assuming an

average human body mass of 65 kg giving a volume of approximately 65 L, the average Cr concentration in the body should be approximately 15 nM. Using 1 mM hydrogen peroxide and letting chromate accumulate for 6 hours, Lay and coworkers observed at best about 30% conversion of any of the Cr(III) compounds examined to chromate. Assuming 30% conversion gives an average chromate concentration of approximately 5 nM. While Cr does accumulate better in some organs than in others such that Cr concentrations locally can be higher than the average, the 5 nM concentration (calculated otherwise assuming the best of all conditions for chromate production) is roughly 15,000 times smaller than the $K_i$ values reported for phosphatase inhibition by chromate. When one remembers that the typical peroxide concentration in the body is $10^{-7}$ to $10^{-8}$ M (rather than mM) and instances where locally higher concentrations of hydrogen peroxide are unlikely to remain for prolonged periods of time, the probability that chromate is responsible for any effects on insulin sensitivity in human studies is essentially nill. (When similar calculations are performed for vanadium supplements being tested for anti-diabetic effects, the calculations are consistent with vanadate potentially being a phosphatase inhibitor, i.e. increased size of the dose, greater % absorption, and much smaller inhibition constants.)

This is not to rule out the possible significance of Cr(IV) or Cr(V) species from the oxidation of Cr(III) supplements in the effects of chromium supplements on insulin sensitivity and related effects. These species as noted by Lay and coworkers [32] could serve to inactivate tyrosine protein phosphatases through oxidation of the active site cysteine thiol. Indeed, insulin action triggers the production of reactive oxygen species including hydrogen peroxide and oxidants can facilitate or mimic insulin action; reactive oxygen species have even been proposed as second messengers in insulin signal transduction, including through inhibition of tyrosine protein phosphatases via thiol oxidation [75]. This, including the relationship of any potential such action to toxicological effects from high valent Cr is an area which warrants additional study. For a more detailed review of the potential significance of this oxidative chemistry, see Chapter 11.

## CYTOKINES

Jain and coworkers have shown that monocytes exposed to high glucose concentrations have lower levels of the cytokine TNF-$\alpha$ (tumor necrosis factor-$\alpha$) in the presence of 100 μM $CrCl_3$ for 24 hours at 37 °C [76]. Treatment with $CrCl_3$ also inhibited stimulation of TNF-$\alpha$ secretion in these cells by 50 μM $H_2O_2$. Lipid peroxidation and protein oxidation in the presence of $H_2O_2$ was also inhibited by $CrCl_3$. As increased TNF-$\alpha$ secretion may be associated with insulin resistance, Jain has proposed that increased insulin sensitivity arising from chromium administration may be mediated by lowering of TNF-$\alpha$ levels [77]. In a follow-up study, this group found that $CrCl_3$ in combination with estrogen lowered lipid peroxidation in high glucose-treated monocytes [78]. The combination was also found to decrease interleukin-6 (IL-6) secretion. Chromium was proposed to potentiate the effects of estrogen (Fig. 4) [78]. Curiously, another group has shown that Cr(III) treatment (350–500 ppm) results in increased TNF-$\alpha$ production by macrophages (in the absence of high glucose concentrations) [79]. This activation by chromium ($CrCl_3$) may be regulated by tyrosine kinases [80]. The results in the presence

of high glucose could also point to an association between reactive oxygen species and chromium, but these studies must be considered extremely preliminary. Studies examining the levels of TNF-$\alpha$, IL-6, estrogen, and related cytokines and hormones in humans administered chromium are required before further conclusion can be drawn. Additionally, the fate of the chromium in these cell culture studies needs to be examined. $CrCl_3$ (actually trans-$[CrCl_2(H_2O)_4]Cl \cdot \times H_2O$) readily hydrolyzes in solution to form hydroxide-bridged oligomers, while Cr(III) can bind to components of the media. Thus, the form of chromium the cells are exposed to may or may not resemble physiologically relevant forms, influencing the results. As with all cell culture studies using chromium compounds, the fate of the chromium introduced needs to be carefully elucidated.

## INSULIN RECEPTOR NUMBER

Insulin receptor number has often been claimed as a potential indicator of Cr deficiency in humans (e.g., [81]). Despite the number of times this claim has been made, this claim is based on only a single study using only seven hypoglycemic subjects; IR number per cell (red blood cell) was increased significantly after 6 weeks but not 12 weeks of Cr treatment [82]. Other studies, including rat studies, have failed to observe any effects on IR number (e.g., [83] but see [84] on statistical analysis of the data).

## CONCLUSION

If chromium is an essential element, the mechanism by which it acts in vivo has not yet been elucidated, although several proposed mechanism of actions based on varying amount of in vitro data exist. As evidence mounts that Cr(III) may have positive pharmacological effects, understanding the mechanism of this action (which may or may not be related to a physiological mechanism) will become increasing important. Devising experiments to test the proposals presented herein or to establish that Cr acts by a completely different mechanism will be a non-trivial enterprise, as is the search for any grail.

## ADDENDUM

After this chapter was completed, Brautigan and coworkers [85] reported results of particular interest to this chapter. Using Chinese hamster ovary cells overexpressing insulin receptor, these workers found that pre-incubation of the cells with chromium picolinate, chromium histidine, or $[Cr_3O(propionate)_6(H_2O)_3]^+$ activated insulin receptor tyrosine kinase activity in the cells at low doses of insulin. While the concentration dependence was only examined for the histidine complex, the effect was concentration dependent. Neither insulin-binding to the cells nor insulin receptor number was affected. Additionally, the addition of chromium did not inhibit dephosphorylation of the insulin receptor by endogenous phosphatases or added PTP1B. Also, chromium apparently did not alter redox regulation of PTP1B (i.e., by trapping the oxidized inactive form or by preventing its reduction and reactivation). $CrCl_3$ and chromium histidine were found not to activate the kinase activity of a recombinant fragment of insulin receptor. The

authors concluded that chromium inside the cell modified the receptor in some manner, activating its kinase activity [85].

## ACKNOWLEDGMENTS

Work on Cr(III) in the laboratory of John B. Vincent has been funded by the NRICGP/USDA, NIH, American Diabetes Association, American Heart Association, and American Chemical Society/PRF. John B. Vincent is also the inventor or co-inventor of five patents on the use of chromium-containing oligopeptides or synthetic Cr(III) compounds as nutritional supplements or therapeutic agents.

## REFERENCES

[1] Saltiel, A. R., and Kahn, C. R. (2001) Insulin signaling and the regulation of glucose and lipid metabolism. *Nature 414*, 799–806.
[2] Goldstein, B. J. (2002) Protein-tyrosine phosphatases: Emerging targets for therapeutic intervention in type 2 diabetes and related states of insulin resistance. *J. Clin. Endocrinol. Metab. 87*, 2474–2480.
[3] Hopkins, L. L., Jr., and Schwarz, K. (1964) Chromium(III) binding to serum proteins, specifically siderophilin. *Biochim. Biophys. Acta 90*, 484–491.
[4] Clodfelder, B. J., Upchurch R. G., and Vincent, J. B. (2004) A comparison of the insulin-sensitive transport of chromium in healthy and model diabetic rats. *J. Inorg. Biochem. 98*, 522–533.
[5] Clodfelder, B. J., Emamaullee, J., Hepburn, D. D., Chakov, N. E., Nettles, H. S., and Vincent, J. B. (2001) The trail of chromium(III) from the blood to the urine: The roles of transferrin and chromodulin. *J. Biol. Inorg. Chem. 6*, 608–617.
[6] Clodfelder, B. J., and Vincent, J. B. (2005) The time-dependent transport of chromium in adult rats from the bloodstream to the urine. *J. Biol. Inorg. Chem. 10*, 383–393.
[7] Dowling, H. J., Offenbacher, E. G., and Pi-Sunyer, F. X. (1990) Effects of plasma transferrin and albumin on the absorption of trivalent chromium. *Nutr. Res. 10*, 1251–1260.
[8] Yamamoto, A., Wada, O., and Ono, T. (1981) A low-molecular-weight, chromium-binding substance in mammals. *Toxicol. Appl. Pharmacol. 59*, 515–523.
[9] Wu, G. Y., and Wada, O. (1981) Studies on a specific chromium binding substance (a low-molecular-weight chromium binding substance) in urine. *Jpn. J. Ind. Health 23*, 505–512.
[10] Wada, O., Manabe, S., Yamaguchi, N., Ishikawa, S., and Yanagisawa, H. (1983) Low-molecular-weight, chromium-binding substance in rat lungs and its possible role in chromium movement. *Ind. Health 21*, 35–41.
[11] Yamamoto, A., Wada, O., and Ono, T. (1984) Distribution and chromium-binding capacity of a low-molecular-weight, chromium-binding substance in mice. *J. Inorg. Biochem. 22*, 91–102.
[12] Yamamoto, A., Wada, O., and Ono, T. (1987) Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. *Eur. J. Biochem. 165*, 627–631.
[13] Davis, C. M., and Vincent, J. B. (1997) Isolation and characterization of a biologically active form of chromium oligopeptide from bovine liver. *Arch. Biochem. Biophys. 339*, 335–343.
[14] Sumrall, K. H., and Vincent, J. B. (1997) Is glucose tolerance factor an artifact produced by acid hydrolysis of low-molecular-weight chromium-binding substance? *Polyhedron 16*, 4171–4177.
[15] Wada, O., Wu, G. Y., Yamamoto, A., Manabe, S., and Ono, T. (1983) Purification and chromium-excretory function of low-molecular-weight, chromium-binding substances from dog liver. *Environ. Res. 32*, 228–239.

[16] Yamamoto, A., Wada, O., and Suzuki, H. (1988) Purification and properties of biologically active chromium complex from bovine colostrum. *J. Nutr. 118*, 39–45.
[17] Dinakarpandian, D., Morrissette, V., Chaudhary, S., Amini, K., Bennett, B., and Van Horn, J. D. (2004) An informatics search for the low-molecular weight chromium-binding peptide. *BMC Chemical Biology 4*:2 (www.biomedcentral.com/1472-6769/4/2).
[18] Vincent, J. B., Hatfield, J., and Burdette, S. (2004) Characterizing chromium-containing oligopeptides. *Abstracts of the 227th ACS National Meeting, Nanheim, CA, March 28–April 1, 2004*, CHED 381.
[19] Kurohara, K., Matsuda, Y., Nagabukuro, A., Tsuji, A., Amagasa, T., and Fujisawa-Sehara, A. (2000) Meltrin b (ADAM 19) gene: Cloning, mapping, and analysis of the regulatory region. *Biochem. Biophys. Res. Commun. 270*, 522–527.
[20] Sun, Y., Ramirez, J., Woski, S. A., and Vincent, J. B. (2000) The binding of trivalent chromium to low-molecular-weight chromium-binding substance (LMWCr) and the transfer of chromium from transferrin and $Cr(pic)_3$ to LMWCr. *J. Biol. Inorg. Chem. 5*, 129–136.
[21] Jacquamet, L., Sun, Y., Hatfield, J., Gu, W., Cramer, S. P., Crowder, M. W., Lorigan, G. A., Vincent, J. B., and Latour, J-M. (2003) Characterization of chromodulin by X-ray absorption and electron paramagnetic spectroscopies and magnetic susceptibility measurements. *J. Am. Chem. Soc. 125*, 774–780.
[22] Levina, A., Codd, R., Dillion, C. T., and Lay, P. A. (2003) Chromium in biology: Toxicology and nutritional aspects. *Prog. Inorg. Chem. 51*, 145–644.
[23] Davis, C. M., and Vincent, J. B. (1997) Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry 36*, 4382–4385.
[24] Gaggelli, E.; Berti, F., D'Amelio, N., Gaggelli, N., Valensin, G., Bovalini, L., Paffetti, A., and Trabalzini, L. (2002) Metabolic pathways of carcinogenic chromium. *Environ. Health Perspect. 110(Suppl. 5)*, 733–738.
[25] Levina, A., Zhang, L. B., and Lay, P. A. (2003) Structure and reactivity of a chromium(V) glutathione complex. *Inorg. Chem. 42*, 767–784.
[26] Yamamoto, A., Wada, O., and Suzuki, H. (1987) Separation of biologically active chromium complex from cow colostrum. *Tohoku J. Exp. Med. 152*, 211–219.
[27] Vincent, J. B. (1994) Relationship between glucose tolerance factor and low-molecular-weight chromium-binding substance. *J. Nutr. 124*, 117–118.
[28] Yamamoto, A., Wada, O., and Manabe, S. (1989) Evidence that chromium is an essential factor for biological activity of low-molecular-weight Cr-binding substance. *Biochem. Biophys. Res. Commun. 163*, 189–193.
[29] Davis, C. M., Sumrall, K. H., and Vincent, J. B. (1996) The biologically active form of chromium may activate a membrane phosphotyrosine phopshatase (PTP). *Biochemistry 35*, 12963–12969.
[30] Davis, C. M., Royer, A. C., and Vincent, J. B. (1997) Synthetic multinuclear chromium assembly activates insulin receptor tyrosine kinase activity: Functional model for low-molecular-weight chromium-binding substance. *Inorg. Chem. 36*, 5316–5320.
[31] Goldstein, B. J., Zhu, L., Hager, R., Zilbering, A., Sun, Y., and Vincent, J. B. (2001) Enhancement of post-receptor insulin signaling by trivalent chromium in hepatoma cells is associated with differential inhibition of specific protein-tyrosine phosphatases. *J. Trace Elem. Exp. Med. 14*, 393–404.
[32] Mulkani, I., Levina, A., and Lay, P. A. (2004) Biomimetic oxidation of chromium(III): Does the antidiabetic activity of chromium(III) involve carcinogenic chromium(VI)? *Angew. Chem. Int. Ed. 43*, 4504–4507.
[33] Davis, C. M., and Vincent, J. B. (1997) Chromium in carbohydrate and lipid metabolism. *J. Biol. Inorg. Chem. 2*, 675–679.
[34] Vincent, J. B. (1999) Mechanism of action of chromium: low-molecular-weight chromium-binding substance. *J. Am. Coll. Nutr. 18*, 6–12.

[35] Meador, W. E., Means, R. A., and Quiocho, F. A. (1992) Target enzyme recognition: 2.4 Å structure of a calmodulin-peptide complex. *Science 257*, 1251–1255.
[36] Morris, B. W., MacNeil, S., Stanley, K., Gray, T. A., and Fraser, R. (1993) The interrelationship between insulin and chromium in hyperinsulinaemic euglycaemic clamps in healthy volunteers. *J. Endocrin. 139*, 339–345.
[37] Anderson, R. A., Polansky, M. M., Bryden, N. A., Roginski, E. E., Patterson, K. Y., Veillon, C., and Glinsmann, W. (1982) Urinary chromium excretion of human subjects: Effects of chromium supplementation and glucose loading. *Am. J. Clin. Nutr. 36*, 1184–1193.
[38] Anderson, R. A., Polansky, M. M., Bryden, N. A., Roginski, E. E., Patterson, K. Y., and Reamer, D. C. (1982) Effect of exercise (running) on serum glucose, insulin, glucagons, and chromium excretion. *Diabetes 31*, 212–216.
[39] Kozlovsky, A. S., Moser, P. B., Reisner, S., and Anderson, R. A. (1986) Effects of diets high in simple sugars on urinary chromium losses. *Metabolism 35*, 515–518.
[40] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Reiser, S. (1990) Urinary chromium excretion and insulinogenic properties of carbohydrates. *Am. J. Clin. Nutr. 51*, 864–868.
[41] Morris, B. M., Blumsohn, A., MacNeil, S., and Gray, T. A. (1992) The trace element chromium – A role in glucose homeostasis. *Am. J. Clin. Nutr. 55*, 989–991.
[42] Morris, B. M., Griffiths, H., and Kemp, G. J. (1988) Effect of glucose loading on concentrations of chromium in plasma and urine of healthy adults. *Clin. Chem. 34*, 1114–1116.
[43] Vincent, J. (2000) The biochemistry of chromium. *J. Nutr. 130*, 715–718.
[44] Sun, Y., Mallya, K., Ramirez, J., and Vincent, J. B. (1999) The biomimetic $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ decreases plasma cholesterol and triglycerides in rats: towards chromium-containing therapeutics. *J. Biol. Inorg. Chem. 4*, 838–845.
[45] Cannon, R. D., and White, R. P. (1988) Chemical and physical properties of triangular bridged metal complexes. *Prog. Inorg. Chem 36*, 195–297.
[46] Nagi, M., Harton, A., Donald, S., Lee, Y. S., Sabat, M., O'Connor, C. J., and Vincent, J. B. (1995) An unsymmetric trinuclear chromium(III) oxo carboxylate assembly: Structure and characterization of $Cr_3O(O_2CPh)_4(8\text{-hqn})_3 1.25CH_2Cl_2$. *Inorg. Chem. 34*, 3813–3820.
[47] Harton, A., Terrell, K., Huffman, J. C., MacDonald, C., Beatty, A., Li, S., O'Connor, C., and Vincent, J. B. (1997) Synthesis and characterization of novel oxo-bridged dinuclear and hydroxo-bridged trinuclear chromium(III) assemblies. *Inorg. Chem. 36*, 4875–4882.
[48] Bino, A., Chayat, R., Pedersen, E., and Schneider, A. (1991) A new tetranuclear chromium(III) complex with a $[Cr_4O_2]$ core: synthesis, structure, and magnetic properties of $[Cr_4O_2(O_2CCH_3)_7(bpy)_2]PF_6$ (bpy = 2,2'-bipyridine). *Inorg. Chem. 30*, 856–858.
[49] Donald, S., Terrell, K., Vincent, J. B., and Robinson, K. D. (1995) Modelling chromium biochemistry: Synthesis and characterization of $[NEt_3H][Cr(III)_4O_2(O_2CPh)_7(pic)_2]\cdot CH_2Cl_2$ (Hpic = Picolinic acid). *Polyhedron 14*, 971–976.
[50] Ellis, T., Glass, M., Harton, A., Folting, K., Huffman, J. C., and Vincent, J. B. (1994) Synthetic models for low-molecular-weight chromium-binding substance: Synthesis and characterization of oxo-bridged tetranuclear chromium(III) assemblies. *Inorg. Chem. 33*, 5522–5527.
[51] Speetjens, J. K., Parand, A., Crowder, M. W., and Vincent, J. B. (1999) Low-molecular-weight chromium-binding substance and biomimetic $[Cr_3O(O_2CH_2CH_3)_6(H_2O)_3]^+$ do not cleave DNA under physiologically-relevant conditions. *Polyhedron 18*, 2617–2624.
[52] Anderson, R. A., Bryden, N. A., and Polansky, M. M. (1997) Lack of toxicity of chromium chloride and chromium picolinate. J. *Am. Coll. Nutr. 16*, 273–279.
[53] Clodfelder, B. J., Gullick, B. M., Lukaski, H. C., Neggers, Y., and Vincent, J. B. (2005) Oral administration of $[Cr_3O(O_2CH_2CH_3)_6(H_2O)_3]^+$ increases insulin sensitivity and improves blood plasma variables in healthy and type 2 diabetic rats. *J. Biol. Inorg. Chem. 10*, 119–130.

[54] Debski, B., Krejpcio, Z., Kuryl, T., Wojciak, R., amd Lipko, M. (2004) Biomimetic chromium(III) complex and fructan supplementation affect insulin and membrane glucose transport in rats. *J. Trace Elem Exp. Med. 17*, 206–207.

[55] Krejpcio, Z., Debski, B., Wojciak, R., Kuryl, T., and Tubacka, M. (2004) Biomimetic chromium(III) complex and fructan supplementation improve blood variables in STZ-induced diabetic rats. *J. Trace Elem Exp. Med. 17*, 207–208.

[56] Shute, A. A., and Vincent, J. B. (2001) The stability of the biomimetic cation triaqua-μ-oxohexapropionatotrichromium(III) in vivo in rats. *Polyhedron 20*, 2241–2252.

[57] Shute, A. A., and Vincent, J. B. (2002) The fate of the biomimetic cation triaqua-μ-oxohexapropionatotrichromium(III) in rats. *J. Inorg. Biochem. 89*, 272–278.

[58] Clodfelder, B. J., Chang, C., and Vincent, J. B. (2004) Absorption of the biomimetic cation triqua-$\mu_3$-oxo-μ-hexapropionatotrichromium(III) in rats. *Biol Trace Elem. Res. 98*, 522–533.

[59] Cefalu, W. T., Martin, J. M., Wachtel, D., Zhang, X. H., and Wang, Z. Q. (2003) Chromium picolinate supplementation increases insulin-stimulated Akt phosphorylation in vivo in skeletal muscle from subjects with type 2 diabetes. 18th International Diabetes Federation Congress, August 24–29, 2003, Paris – France, Abstract 154.

[60] Cefalu, W. T. (2003) "Mechanism of action of chromium: Cellular signaling", presentation at Chromium in Health and Disease, A CADRE Research Summit, April 1–2, 2003, Boston, MA.

[61] Cefalu, W. T., Wang, Z. Q., Zhang, X. H., Baldor, L. C., and Russell, J. C. (2002) Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle Glut-4 translocation in obese, hyperinsulinemic (JCR-LA) rats. *J. Nutr. 132*, 1107–1114.

[62] Yang, X., Palanichamy, K., Ontko, A. C., Rao, M. N. A., Fanf, C. X., Ren, J., and Sreejayan, N. (2005) A newly synthetic chromium complex – chromium(phenylalanine)$_3$ improves insulin responsiveness and reduces whole body glucose tolerance. *FEBS Lett. 579*, 1458–1464.

[63] Levina, A., and Lay, P. A. (2005) Mechanistic studies of relevance to the biological activities of chromium. *Coord. Chem. Rev. 249*, 281–298.

[64] Kim, E., and Na, K. J. (1991) Effect of sodium dichromate on carbohydrate metabolism. *Toxicol. Appl. Pharmacol. 110*, 251–258.

[65] Goto, Y., and Kida, K. (1995) Insulin-like action of chromate on glucose transport in isolated rat adipocytes. *Jpn. J. Pharmacol. 67*, 365–368.

[66] Yurkow, E. J., and Kim, G. (1995) Effects of chromium on basal and insulin-induced tyrosine phosphorylation in H4 hepatoma cells: comparison with phorbol-12-myristate-13-acetate and sodium orthovanadate. *Mol. Pharmacol. 47*, 686–695.

[67] Kim, G., and Yurkow, E. J. (1996) Chromium induces a persistent activiation of mitogen-activated protein kinases by a redox-sensitive mechanism in H4 rat hepatoma cells. *Cancer Res. 56*, 2045–2051.

[68] Yang, L., Crans, D. C., Yuen, V. G., McNeill, J., and Willsky, G. R. (2000) Chemistry and insulin-like properties of chromium 2,6-pyridinedicarboxylate complexes. Abstracts of Papers – American Chemical Society, 220th, 2000, INOR-047.

[69] Thompson, K. H., Chiles, J., Yuen, V. G., Tse, J., McNeill, J. H., and Orvig, C. (2004) Comparison of anti-hyperglycemic effect amongst vanadium, molybdenum and other metal maltol complexes. *J. Inorg. Biochem. 98*, 683–690 and references therein.

[70] Peters, K. G., Davis, M. G., Howard, B. W., Pokross, M., Rastogi, V., Diven, C., Greis, K. D., Eby-Wilkens, E., Maier, M., Evdokimov, A., Soper, S., and Genbauffe, F. (2003) Mechanism of insulin sensization by BMOV (bis maltolato oxo vanadium); unligated vanadium ($VO_4$) as the active component. *J. Inorg. Biochem. 96*, 321–330.

[71] Goldfine, A. B., Patti, M. E., Zuberi, L., Goldstein, B. J., LeBlanc, R., Landaker, E. J., Jiang, Z. Y., Willsky, G. R., and Kahn, C. R. (2000) Metabolic effects of vanadyl sulfate in humans

with non-insulin dependent diabetes mellitus: in vivo and in vitro studies. *Metabolism 49*, 400–410.

[72] Lu, B., Ennis, D., Lai, R., Bogdanovic, E., Nikolov, I. G., Salamon, L., Fantus, C., Le-Tien, H., and Fantus, I. G. (2001) Enhanced sensitivity of insulin-resistant adipocytes to vanadate is associated with oxidative stress and decreased reduction of vanadate(+5) and vanadyl(+4). *J. Biol. Chem. 276*, 35589–35598.

[73] Olin, K. L., Stearns, D. M., Armstrong, W. H., and Keen, C. L. (1994) Comparative retention/absorption of chromium ($^{51}$Cr) from $^{51}$Cr chloride, $^{51}$Cr nicotinate and $^{51}$Cr picolinate in a rat model. *Trace Elem. Electrolytes 11*, 182–186.

[74] Anderson, R. A., Bryden, N. A., Polansky, M. M., and Gautschi, K. (1996) Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. *J. Trace Elem. Exp. Med. 9*, 11–25.

[75] Goldstein, B. J., Kalyankar, M., and Wu, X. (2005) Redox paradox: Insulin action is facilitated by insulin-stimulated reactive oxygen species with multiple potential signaling targets. *Diabetes 54*, 311–321.

[76] Jain, S. K., and Kannan, K. (2001) Chromium chloride inhibits oxidative stress and TNF-α secretion caused by exposure to high glucose in cultured U937 monocytes. *Biochem. Biophys. Res. Commun. 289*, 687–691.

[77] Kordella, T. (2004) Chromium:Can it help kids with type 2? *Diabetes Forecast 57*, 83–85.

[78] Jain, S. K., Rogier, K., Prouty, L., and Jain, S. K. (2004) Protective effects of 17β-estradiol and trivalent chromium on interleukin-6 secretion, oxidative stress, and adhesion of monocytes: Relevance to heart disease in postmenopausal women. *Free Radic. Biol. Med. 37*, 1730–1735.

[79] Catelas, I., Petit, A., Zukor, D. J., Antoniou, J., and Huk, O. L. (2002) TNF-α secretion and machrophage mortality induced by cobalt and chromium ion in in vitro – Qualitative analysis of apoptosis. *Biomaterials 24*, 383–391.

[80] Luo, L., Petit, A., Antoniou, J., Zukor, D. J., Huk, O. L., Liu, R. C. W., Winnik, F. M., and Mwale, F. (2005) Effect of cobalt and chromium ions on MMP-1, TIMP-1, and TNF-α gene expression in human U937 macrophages: A role for tyrosine kinases. *Biomaterials*, *26*, 5587–5593.

[81] Anderson, R. A. (1989) Essentiality of chromium in humans. *Sci. Total Environ. 86*, 75–81.

[82] Anderson, R. A., Polansky, M. M., Bryden, N. A., Bhathena, S. J., and Canary, J. J. (1987) Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. *Metabolism 36*, 351–355.

[83] Yoshimoto, S., Sakamoto, K., Wakabayashi, I., and Masui, H. Effect of chromium on glucose tolerance in stroke-prone spontaneously hypertensive rats with streptozotocin-induced diabetes. (1992) *Metabolism 41*, 636–642.

[84] Vincent, J. B. (2001) The bioinorganic chemistry of chromium(III). *Polyhedron 20*, 1–26.

[85] Wang, H., Kruszewski, A., and Brautigan, D. L. (2005) Cellular chromium enhances activation of insulin receptor kinase. *Biochemistry*, *44*, 8167–8175.

**Part III**

# Chromium(III) as a therapeutic agent

This page intentionally left blank

*Chapter 8*

# Clinical effect of chromium supplements on human health

**William T. Cefalu**

Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Road, Baton Rouge, LA 70808

## INTRODUCTION

The primary clinical strategy employed by health care providers to improve risk factors for human disease, particularly those for cardiovascular disease (CVD) and diabetes, consists of lifestyle modification combined with pharmacologic intervention [1]. However, alternative strategies, for example nutritional supplementation with over-the-counter agents, are extensively practiced by a large number of patients with chronic diseases and are frequently undertaken without first informing the medical provider. According to the FDA, there are more than 29,000 different nutritional supplements available to consumers, and estimates are that Americans spend over 12 billion per year on these supplements [2, 3]. Unfortunately, considerable controversy exists regarding the use of dietary supplements in human health because efficacy data for many of the supplements consists of only uncontrolled studies and anecdotal reports. As such, there is a paucity of data in humans in regard to the effect of most commercially available supplements to improve metabolic abnormalities.

One supplement that has attracted considerable clinical interest, particularly as it relates to carbohydrate metabolism, is chromium (Cr) [4]. The interest in chromium supplementation as a clinical intervention to enhance carbohydrate metabolism had its origins in the 1950s. Specifically, at that time, research observations suggested that brewer's yeast contained a glucose tolerance factor (GTF) that was effective to improve glucose levels in experimental animals [5]. Ultimately, this "factor" was eventually suggested to be a biologically active form of trivalent chromium as it substantially improved glucose metabolism in diabetic mice [6]. The interest for chromium supplementation in patients with diabetes was also heightened by the clinical observations made in the 1970s for patients receiving total parenteral nutrition (TPN), demonstrating that chromium was an essential nutrient required for normal carbohydrate metabolism. Specifically, it was demonstrated that individuals receiving TPN over time may develop symptoms of diabetes which were refractory to increasing insulin dosing [7]. Supplemental chromium was provided which resulted in markedly improved glycemic status and greatly reduced

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

insulin requirements. Other studies suggested similar effects in patients on TPN [8]. As such, chromium is now routinely added to TPN solutions [9]. The results of these studies strongly implicated chromium as a critical cofactor in the action of insulin [10, 11].

As suggested from the above discussion, chromium replacement in deficiency states is well established. However, the role of chromium supplementation to enhance glucose metabolism in subjects, not felt to be chromium deficient, remains controversial. Routine use of Cr supplementation, particularly for those individuals with diabetes, is not currently recommended. Specifically, the clinical practice recommendations from the American Diabetes Association for the year 2005 state that "the existence of a relationship between chromium picolinate and either insulin resistance or Type 2 diabetes was highly uncertain" [12]. Interestingly, these recommendations have not deterred its use and Cr supplementation by the general public, and in subjects with diabetes in particular, has surpassed our ability as a scientific community to provide evidence regarding its safety and efficacy. Despite widespread use by patients with diabetes and anecdotal reports in the past regarding its efficacy, until recently, data in humans concerning chromium's effects on insulin action in vivo or on cellular aspects of insulin action were scarce. Conflicting data have been reported that has contributed greatly to the confusion among health care providers regarding the effect of chromium supplementation on parameters assessing human health. Furthermore, elucidating the cellular and molecular mechanisms by which chromium supplements affect carbohydrate metabolism in vivo is necessary before specific recommendations can be made regarding its routine use in the management of diabetes.

A review of the literature that evaluated the role of chromium supplementation on parameters assessing glucose metabolism only served to provide more confusion for health care providers regarding routine use of chromium in diabetic states (Table 1) [13, 14–16, 17–27]. As demonstrated in Table 1, multiple studies that evaluated chromium supplementation in various populations reported considerable differences in efficacy on the various parameters assessing carbohydrate metabolism. These earlier studies may be difficult to interpret due to many factors and limitations, for example study design, patient selection, dosage, and formulation [28]. First and foremost, the appropriate study design for clinical research trials evaluating a specific intervention is of utmost importance. The use of a control group with a double-blind design is required given the possibility that patients who choose to use chromium may be different from nonusers. As such, it would appear that only a randomized intervention can definitely establish the overall effects of chromium on carbohydrate metabolism. It is this design that controls for biases, whether known or unknown, that may confound the association and assessment of chromium supplementation and carbohydrate metabolism. Unfortunately, many of the studies reported in Table 1 were open label and therefore generated substantial bias. A second limitation of the earlier studies was the availability and/or choice of the laboratory or clinical parameter used to assess response on carbohydrate metabolism. The use of fasting blood glucose levels, glucose tolerance tests, or mixed-meal tests are valuable parameters and frequently reported for outpatient clinical studies. Unfortunately, these tests do not provide the sensitivity to precisely assess specific parameters such as whole body insulin action. The gold standard for assessing insulin action, that is the hyperinsulinemic–euglycemic clamp, did not appear to have been used in the majority of the studies reported. One study [29] did use the euglycemic clamp to assess the

Table 1
Comparison of chromium studies in humans. Table modified from Cefalu, et al. [4]

| Reference | Study type | Study length | Chromium supplement and dose | Subjects | N | Technique assessed | Results | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Glucose | Insulin | HbA1c | IS |
| *Studies using CrCl, brewer's yeast, and CrN formulation* | | | | | | | | | | |
| Trow [17] | OL | 2 months | Brewer's yeast, (100 μg/day $Cr^{3+}$) | Type 2 | 12 | OGTT | – | – | NA | NA |
| Sherman [18] | DB | 4 months | $CrCl_3$, 150 μg/day | Type 2/1/ Nondiabet | 14 | OGTT | – | NA | NA | NA |
| Rabinowitz [16] | DB | 4 months | $CrCl_3$, 150 μg/day; Brewer's yeast (13 μ/day $Cr^{3+}$) | Type 2 | 43 | Meal challenge | – | – | NA | NA |
| Uusitupa [14] | DB | 6 months | Brewer's yeast (160 μg/day $Cr^{3+}$) | Impaired Tolerant, Elderly | 26 | OGTT | – | – | – | NA |
| Uusitupa [19] | DB | 6 weeks | $CrCl_3$, 200 μg/day | Type 2 | 10 | OGTT, HbA1c | – | ↓ | – | NA |
| Potter [13] | OL | 3 months | $CrCl_3$, 200 μg/day | Impaired Tolerant | 5 | Hyperglycemic clamp | – | – | – | ↑ |
| Mossop [20] | DB | 3 months | $CrCl_3$, 600 μg/day | Type 1 / 2 | 26 | FBG | ↓ | NA | NA | NA |
| Nath [21] | OL | 2 months | $CrCl_3$, 500 μg/day | Type 2 | 12 | OGTT | ↓ | ↓ | NA | NA |
| Glinsmann [22] | OL | 18–133 days | $CrCl_3$, 180–3000 μg/day | Type 1; Type 2 | 6 | IVGTT, OGTT | ↓ | NA | NA | NA |
| Wilson [15] | DB | 3 months | CrN 220 μg/day | Nondiabetic young | 26 | FBG/insulin | – | ↓[a] | NA | NA |
| *Studies using CrPic formulation* | | | | | | | | | | |
| Anderson [34] | R | 4 months | CrPic, 200 or 1000 μg/day | Type 2 | 180 | OGTT, HbA1c | ↓ | ↓ | ↓ | NA |

(*continued*)

Table 1
(Continued)

| Reference | Study type | Study length | Chromium supplement and dose | Subjects | N | Technique assessed | Results | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Glucose | Insulin | HbA1c | IS |
| Amato [23] | DB | 2 months | CrPic, 1000 μg/day | Nondiabetic elderly | 19 | Minimal model | – | – | NA | – |
| Jovanovic [24] | DB | 2 months | CrPic, 320 or 640 μg/day* | Gest diabetes | 20 | OGTT, HbA1c | ↓ | ↓ | – | NA |
| Ravina [25] | OL | 1–7 days | CrPic, 600 μg/day | Diabetes | 3 | FBG | ↓ | NA | NA | NA |
| Morris [26] | OL | 3 months | CrPic, 400 μg/day | Type 2 | 5 | Insulin tolerance, HOMA | – | ↓ | NA | ↑ |
| Cheng [27] | OL | 1–10 months | CrPic, 500 μg/day | Type 2 | 833 | Fasting, post-meal | ↓ | NA | NA | NA |
| Ghosh [35] | DB | 3 months | $Cr_3$, 200 μg/day | Type 2 | 50 | FBG, HbA1c | ↓ | NA | ↓ | NA |
| Cefalu [30] | R | 8 months | CrPic, 1000 μg/day | Pre-diabetes | 29 | Minimal model | – | ↓ | NA | ↑ |
| Ravina [36] | OL | 10 days | CrPic, 200 μg/day | Type 1/Type 2 | 48/114 | Insulin tolerance, HbA1c | NA | NA | ↓ | ↑ |
| Lee [41] | DB | 2 months | CrPic, 200 μg/day | Type 2 | 30 | FBG, HbA1c | – | NA | – | NA |
| Evans [71] | DB | 42 days | CrPic, 200 μg/day | Type 2 | 11 | FBG, HbA1c | ↓ | NA | ↓ | NA |

OL = Open label; OGTT = Oral glucose tolerance test; ↓ = Decreased; DB = Double-blind;
* = β-cell sensitivity to glucose; IS = Insulin sensitivity; ↑ = Increased U/A = Urinanalysis;
a = In hyperinsulinemic patients only; FBG = Fasting blood glucose; – = No change;
Y = Hair, RBCs, plasma, urinanalysis; NA = Not assessed.

relationship of blood chromium and insulin, and another study [13] evaluated subjects with a hyperglycemic clamp and demonstrated a significant increase in $\beta$-cell sensitivity to glucose following chromium supplementation. Several other studies used either the minimal model technique, the insulin tolerance test or the homeostasis model assessment method [26, 30]. A third concern for the previously reported studies was that the characteristics of the subjects evaluated varied tremendously, even within the same study. For example, several studies grouped type 1 and type 2 diabetic subjects together in the evaluation of chromium's effect. Clearly, the pathophysiology of these conditions is markedly different. Even in studies in which only subjects with type 2 diabetes were reported, subjects were assessed while on various therapies (e.g., diet, sulfonylureas, metformin, insulin) and at different levels of glycemic control [16, 17, 22, 25, 27]. Clinical factors such as hyperglycemia, in addition to the different mechanisms of action from oral hypoglycemic agents, will provide significant confounders when assessing the effect of chromium supplementation on insulin action [31–33]. A fourth concern is related to the dosage, formulation, and length of observation. The amount of time subjects were maintained on chromium supplements ranged greatly (ranging from 1 day to 8 months). The dose used (ranging from 100 to 3,000 μg daily) also varied tremendously in the reported studies. It appeared that studies evaluating $\leq 200$ μg of Cr as chromium chloride failed to elicit a consistent clinical response in those with type 2 diabetes, although one study did demonstrate a positive effect at 200 μg of Cr as CrCl salt [19]. More consistent clinical responses were observed when subjects consumed a daily dose of chromium $>200$ μg/day, and maintained that dose for a duration of $>2$ months (Table 1). It has been suggested that other forms of chromium, such as chromium picolinate, may be more bioavailable and clinically more effective than chromium chloride in both human and animal studies. Finally, none of the earlier studies addressed the role of chromium blood levels, or change in levels, on the measured parameters. For future studies, these limitations will need to be addressed in clinical research trials so that a better understanding of the clinical effect of chromium can be assessed.

## EFFECT OF CHROMIUM SUPPLEMENTATION REPORTED FOR SPECIFIC POPULATIONS

### Individuals with type 1 and 2 diabetes

One of the largest studies to date assessing chromium supplementation in individuals with type 2 diabetes was a double-blind, placebo-controlled study involving 180 subjects. In that study, Cr effects were reported to be greater at 1000 μg/day than at 200 μg/day [34] (Fig. 1). The most dramatic improvements were shown in hemoglobin $A_{1C}$, which is a reliable indicator of long-term glucose control. Depending on the specific assay used, the upper limit for the hemoglobin $A_{1C}$ is generally $<6.5\%$. Hemoglobin $A_{1C}$ in the placebo group was $8.5 \pm 0.2\%$, $7.5 \pm 0.2\%$ in the 200 μg group, and $6.6 \pm 0.1\%$ in the group of subjects receiving 1000 μg of Cr as chromium picolinate per day for 4 months. Similar results were observed in a recent double-blind, placebo-controlled, crossover study involving 50 subjects with type 2 diabetes supplemented with 200 μg of Cr twice daily as chromium picolinate [35]. An additional study reported that 10 days of treatment with chromium picolinate (CrPic) at 200 μg/day significantly increased insulin sensitivity in patients with type 1 or 2 diabetes and also permitted reductions in

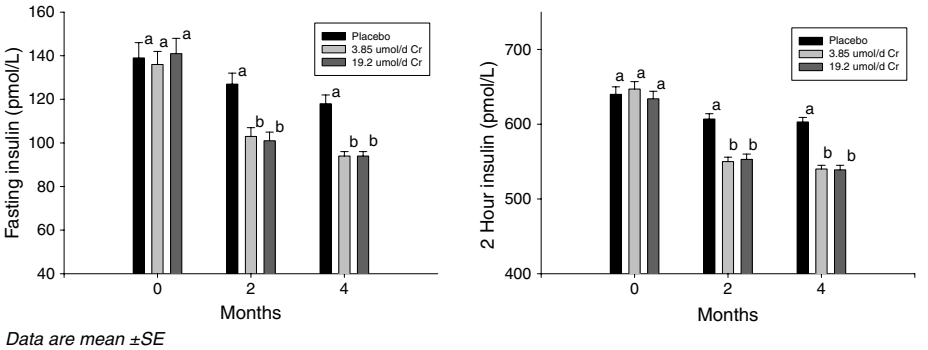



Fig. 1. Supplemental chromium effects on fasting (a) and 2-hr insulin concentrations (b) [34].

dosages of insulin and/or oral antidiabetic drugs in these patients [36]. In addition to these studies, a large long-term study showed that 10 months of treatment with CrPic at a dose of 500 μg/day in 833 patients with type 2 diabetes significantly improved both fasting and postprandial plasma glucose levels versus baseline and reduced the incidence of diabetes symptoms, including fatigue, thirst, and frequent urination [27].

Additional studies using different sources and formulations of chromium, for example brewer's yeast (23.3 μg Cr/day) and chromium chloride (200 μg Cr/day), have been reported. These studies evaluated the effect of the added nutritional supplement on glucose tolerance, serum lipids, and antidiabetic drug dosage in a 16-week, randomized, double-blind, crossover trial that included 78 patients with type 2 diabetes [37, 38]. Both forms of chromium supplementation resulted in significant decreases in glycemia as assessed by mean fasting and 2h glucose, and fructosamine, a marker of short-term protein glycation. Additional studies have been reported for jiangtangkang (8 g t.i.d.), a chrysanthemum product high in chromium, on glucose and insulin metabolism in 188 patients with type 2 diabetes [39]. After 2 months, jiangtangkang treatment reduced fasting and postprandial blood glucose and $HbA_{1c}$ without any corresponding change in plasma insulin. A 16-week, double-blind, randomized, crossover trial of chromium chloride, brewer's yeast that contained chromium as GTF, brewer's yeast extract without GTF, and a placebo was conducted in 43 patients with diabetes [16]. This study was interesting in that although positive effects of chromium on glucose and insulin metabolism were not observed (specifically, fasting glucose and the glucose response to either a standard meal or tolbutamide were not significantly altered by any of the treatments), ketosis-resistant patients experienced a significant increase in postprandial insulin after treatment with the brewer's yeast that contained GTF.

Despite the positive effects of chromium on clinical glycemia as reported from the above-mentioned studies, not all studies have demonstrated significant positive effects of chromium supplementation in patients with diabetes. For example, in a study of 76 patients with ages ranging from 42 to 83 years and with atherosclerotic disease for which 25 were reported to have type 2 diabetes, chromium supplementation for 7–16 months at 250 μg/day versus placebo did not significantly affect serum glucose

levels [40]. An additional study evaluated the effect of chromium supplementation (200 μg/day for 2 months) versus placebo on blood glucose and $HbA_{1c}$ in 30 patients with type 2 diabetes and found no significant effects [41]. Similarly, another study reported that 6 weeks of supplementation with 200 μg/day chromium in 10 patients with type 2 diabetes was not significantly different from placebo in improving glucose tolerance or fasting or 2-hour serum insulin. However, 1-h serum insulin was significantly lower with chromium supplementation than with placebo [19].

What are the differences in those studies that show a positive effect from chromium supplementation versus those in which a negative effect was seen? As discussed above, past studies evaluated different populations for variable periods of time. In addition, the lack of significant effects of chromium supplementation in these three studies may be related to the relatively low chromium doses and specific formulations used for treatment as discussed above. Abraham et al. [40] treated patients with 250 μg $CrCl_3$/day; Lee and Reasner [41] administered 200 μg CrPic/day; and Uusitupa, et al. [19] treated patients in their trial with 200 μg $CrCl_3$/day. Thus, two of the three studies that failed to document significant positive effects of chromium on insulin or glucose metabolism reported using an inorganic formulation that may have been poorly absorbed. The third study used a lower dose of chromium as the picolinate formulation. These facts underscore the point that the chromium formulation used and the dose suggested must be carefully considered when evaluating results from studies that have assessed its metabolic effects in individuals with or without diabetes.

**Gestational diabetes**

Diabetes can complicate pregnancy in many ways. If diabetes is present and not adequately controlled before conception, morbidity during pregnancy is clearly increased. However, diabetes during pregnancy, generally in the last trimester, is referred to as gestational diabetes. Women with gestational diabetes may revert back to euglycemia after delivery, but have a high rate of progression to type 2 diabetes in subsequent years, particularly if the individual develops obesity. Very few studies have evaluated the use of chromium supplementation in gestational diabetes. But, it was demonstrated in a placebo-controlled study of 30 women with gestational diabetes treated with either 4 or 8 μg/kg chromium picolinate or placebo that 8 weeks of chromium supplementation significantly decreased fasting levels of glucose, insulin, and C-peptide versus placebo [24].

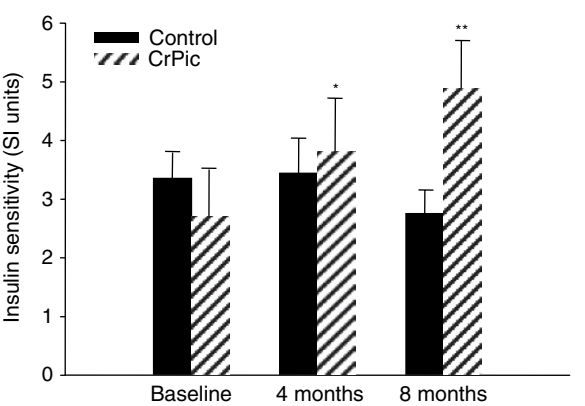
**Steroid-induced diabetes**

It is well established that clinical use of steroids can exacerbate hyperglycemia by worsening insulin resistance. Ravina et al. [25] showed that administration of chromium can also reverse corticosteroid-induced diabetes in a study of only a few patients. Specifically, they treated three patients with steroid-induced diabetes with 600 μg CrPic/day and reported that fasting blood glucose values fell from 250 to 150 mg/dL. The requirement for antidiabetic drugs was also reduced by 50% in these patients.

**Individuals with the metabolic syndrome and impaired glucose tolerance**

Many individuals have additional metabolic abnormalities that, taken together, constitute what has been referred to as the metabolic syndrome. “Metabolic syndrome” describes the human condition characterized by the presence of co-existing traditional

risk factors for CVD such as hypertension, dyslipidemia, glucose intolerance, obesity, and insulin resistance, in addition to non-traditional CVD risk factors such as inflammatory processes and abnormalities of the blood coagulation system [42–47]. Although the etiology for metabolic syndrome is not specifically known, it is well established that obesity and insulin resistance are generally present. Insulin resistance, defined as a clinical state in which a normal or elevated insulin level produces an impaired biologic response, is considered to be a hallmark for the presence of metabolic syndrome [48]. Insulin resistance can be secondary to rare conditions such as abnormal insulin molecules, circulating insulin antagonists (e.g. glucocorticoids, growth hormone, anti-insulin antibodies), or even secondary to genetic syndromes such as the muscular dystrophies [48]. However, the insulin resistance considered as part of the metabolic syndrome essentially represents a target-tissue (i.e., skeletal muscle) defect in insulin action and accounts for the overwhelming majority of cases of insulin resistance reported for the human condition [42–48]. The cellular mechanisms that contribute to insulin resistance are not fully understood. Due the fact that insulin resistance is a key feature of the obesity and metabolic syndrome and that chromium is proposed to modulate insulin action, there has been interest in the role of chromium supplementation in these clinical states. For example, the effect of chromium supplementation was assessed in 29 subjects with >125% of ideal body weight and a family history of diabetes. In that study, Cefalu et al. [30] demonstrated that 8 months of treatment with CrPic (1,000 μg/day) when compared to placebo significantly improved insulin sensitivity as assessed with the modified minimal model technique (Fig. 2). The observation was independent of significant effects on body weight, abdominal fat, or BMI, suggesting a direct effect of chromium on muscle insulin action. In contrast, no significant changes in glucose or insulin metabolism versus placebo were reported after 6 months of treatment with $Cr^{3+}$-rich yeast (160 μg/day) in a group of 26 elderly subjects with impaired glucose tolerance and moderate obesity




*p < 0.05, **p < 0.005, vs. baseline;
Data are means +SD.

Fig. 2. Insulin sensitivity [30].

(BMI ~30 kg/m$^2$ at baseline) [14]. A third study which randomized 40 individuals with impaired glucose tolerance to either placebo or 800 μg/day of chromium picolinate failed to demonstrate an effect on glucose tolerance, insulin sensitivity, or lipid profile [49]. However, there was some question as to the exact amount of elemental chromium provided for that study. For example, chromium picolinate administered in the study at 800 μg per day was suggested to yield a daily dose of 100 μg per day of elemental chromium (i.e., chromium picolinate contains 12.4% elemental chromium). An elemental chromium dose of 100 μg a day was half of the suggested minimum amount (200 μg) of elemental chromium previously shown to exhibit efficacy in glucose and lipid metabolism. A daily dose of 200–1000 μg of elemental chromium, as chromium picolinate, is the efficacious dosage range used in previous studies (see Table 1). Thus, the study may have used a dose that was lower than studies showing a positive effect on glucose metabolism in other reported studies [50]. Two additional studies that evaluated chromium supplementation in subjects with Type 2 diabetes reported different results. In the study of Martin et al., improved insulin sensitivity with 1000 μg/d of Cr as CrPic was reported in individuals with Type 2 diabetes while maintained on sulfonylurea therapy [82]. However, Kleefstra et al. [83] evaluated 1000 ug of Cr as CrPic and reported no significant benefit in obese patients with Type 2 diabetes. The patient selection from the study of Kleefstra differed greatly from the study from Martin et al. [82]. Subjects from the study of Kleefstra et al. were more obese (BMI means ranging 33–35) and were on high dose insulin therapy (mean daily dose ranged 78–105 units/day). In addition, subjects were more advanced in their disease process (mean diabetes duration ranged 10.9–18.4 years). Finally, subjects were taking other medications in addition to insulin. Therefore, based on the above factors including duration of observation, higher dose of elemental Cr, subject selection and techniques assessed, the two studies differed greatly.

**Individuals without diabetes**

There have been many studies that have assessed the effect of chromium supplementation on carbohydrate metabolism in individuals without diabetes. No significant treatment effect on insulin sensitivity was observed for a small-scale study that included 19 nonobese elderly subjects treated with 1000 μg/day CrPic or placebo for 8 weeks [23]. In another study, administration of 400 μg/day chromium for 12 weeks in 44 moderately obese middle-aged women who were also participating in a weight-training and walking program was reported to have no significant effects versus placebo on fasting plasma glucose, serum insulin, plasma glucagon, or serum C-peptide [51]. Chromium supplementation (220 μg/day chromium delivered as chromium nicotinate) also had no significant effect versus placebo on fasting glucose or immunoreactive insulin in 26 young volunteers. However, chromium administration did significantly reduce immunoreactive insulin levels in subjects with baseline concentrations >35 pmol/l [15]. In contrast, results from another trial in which 24 elderly subjects (8 with diabetes) were treated for 8 weeks with either 9 g/day $Cr^{3+}$-rich brewer's yeast or $Cr^{3+}$-poor *Torula* yeast indicated that the $Cr^{3+}$-rich supplement significantly improved glucose tolerance and decreased insulin output [52].

### Meta-analysis of reported trials

Due to the conflicting data reported for the various trials, there have been attempts to analyze all studies using common parameters to determine if a consistent message can be made. As such, a meta-analysis of published studies on the effects of Cr on glucose and insulin has been reported [53]. Based on the data collected and analyzed, the authors concluded that chromium had no significant effects on carbohydrate metabolism. It appears, however, that several studies demonstrating positive effects were not included in the analysis due to lack of specific data and inability to have access to the original data. Those studies that reported no beneficial effects of supplemental Cr generally evaluated healthy normal subjects with good glucose tolerance who would not be expected to respond to additional Cr [54]. Response to Cr on carbohydrate metabolism appears to depend upon the amount and form of Cr consumed and studies involving 200 μg of Cr or less or a form of Cr that is poorly absorbed may not demonstrate effects of supplemental Cr [54]. Other possible shortcomings of the meta-analysis study have also been cited [55].

## CLINICAL EFFECT OF CHROMIUM SUPPLEMENTATION MAY DEPEND ON PHENOTYPE

There is evidence suggesting that a specific phenotype,that is obesity, responds better to chromium supplementation as has been demonstrated in animal studies. Specifically, a phenotype effect has been observed in the JCR: LA-cp rat, an animal model of insulin resistance and obesity. In previous studies, we observed that lean JCR rats were much more insulin sensitive than the lean rats, and insulin sensitivity was not increased further in the lean rats administered CrPic versus control [56] (Fig. 3). However, the attenuation in insulin action in the obese, insulin resistant JCR rats was partially restored with CrPic. Therefore, a very relevant question would be whether hyperinsulinism, insulin resistance,

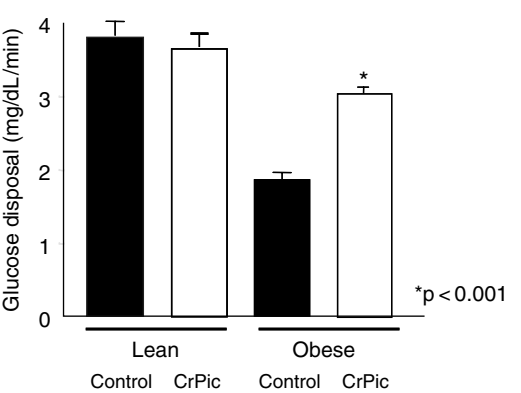



Fig. 3. Demonstrates glucose disposal measures in both lean and obese JCR:LA-cp rats. As observed, obese rats had attenuated glucose disposal compared to lean rats. CrPic significantly increased glucose disposal in obese rats compared to obese controls. Data are mean ±SE.

and/or obesity plays a role in Cr metabolism and/or excretion. Such an observation, if validated, may partially explain the reported discrepancies in response to Cr in the human population and why Cr supplementation appears to have a more predictable response in hyperinsulinemic or obese states.

The in vivo data observed with the use of chromium in these animal studies appears to correlate well to specific cellular signaling data. Cellular signals in muscle, such as insulin receptor substrate-1 (IRS-1) phosphorylation and PI-3 kinase activity, did not differ in the lean JCR rats administered CrPic when compared to controls [57]. However, obese JCR rats had reduced IRS phosphorylation and activation of PI-3 kinase after insulin stimulation when compared to lean controls. Both of these cellular pathways were partially restored in obese rats administered CrPic, but not to levels seen in the lean rats [57] (Fig. 4). Finally, there was no difference in PTP1B levels or activity in skeletal muscle from lean rats as no attenuation in insulin action was observed in vivo. The effect of CrPic to modulate insulin action and PTP1B activity was seen entirely in the obese rats. Thus, it appears that in insulin-resistant states the abnormalities in insulin action are partially reversible with chromium supplementation. However, in insulin sensitive states where the abnormalities are not present, chromium had no observable effect [57].

## EFFECT OF CHROMIUM SUPPLEMENTATION ON BODY WEIGHT AND COMPOSITION

Obesity significantly increases the risk for development of type 2 diabetes, hypertension, and CVD, and, unfortunately, the prevalence of obesity is increasing throughout the world. Currently it is estimated that approximately 7–8% of the population in the United States suffers from the complications of adult-onset diabetes, and it has been estimated that approximately 40% are obese and may have the metabolic syndrome [58–60].

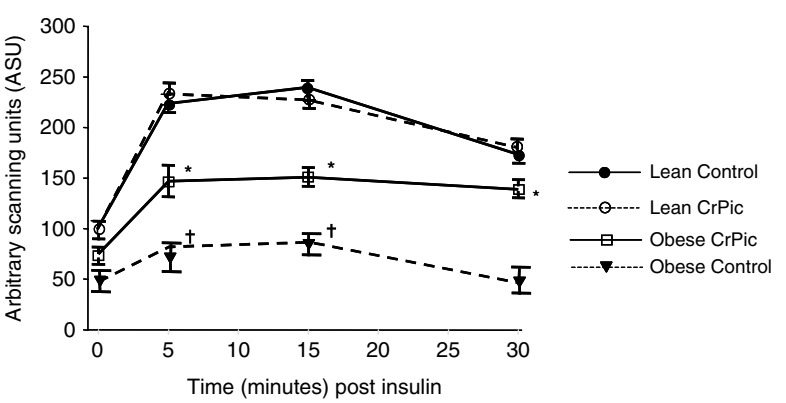



Fig. 4. IRS-1 associated PI-3 Kinase activity for all obese and lean rats. CrPic did not enhance IRS-1 associated PI-3 kinase activity in CrPic supplemented lean rats vs lean controls. Obese rats had attenuated activity vs lean controls. However, obese rats randomized to CrPic had Significantly increased activity vs obese controls (* $p < 0.001$ vs obese control; † $p < 0.001$ vs lean control). Data are mean ±SE.

Minority ethnic groups are at even greater risk. Therefore, it is not surprising that the World Health Organization has listed these conditions as one of the top ten global health problems in Western cultures, and some have considered it the most dangerous disease in the world today [61, 62]. Although, it has been shown that lifestyle interventions consisting of weight loss and exercise will greatly improve insulin sensitivity and can delay the progression to type 2 diabetes, maintenance of lifestyle changes in humans over a long-term period is poor. Thus, use of a biologically based therapy, such as Cr, that may improve pathogenic mechanisms, combined with the acceptance and widespread use by the general public, represents a very attractive, novel, and potentially effective approach to the problem.

Chromium supplementation has been reported to have variable effects on body weight and composition in patients with diabetes [14, 23, 34, 51, 63–68]. One study conducted in patients with diabetes indicated no significant effects on either body weight or BMI [34], while another conducted in elderly subjects with impaired glucose tolerance demonstrated significant reductions in BMI [14]. Of the eight double-blind, placebo-controlled trials in individuals without diabetes, chromium supplementation showed decreases in weight and fat in three larger studies [23, 51, 63–68]. A recent meta-analysis reported a body weight reduction of 1.1–1.2 kg during an intervention period of 10–13 weeks of Cr supplementation, too small to be clinically significant [69]. These results generally support the view that chromium supplementation has at best modest effects on body weight or composition in individuals with diabetes and perhaps more consistent positive effects in healthy volunteers. However, it must be noted that most of the studies addressing this question included only small numbers of subjects and were of relatively short duration. Thus, it is imperative that long-term administration studies be performed to determine whether the reduction in body weight can be maintained [69]. It may take more than 6 months to detect changes in lean body mass in humans, and there may not be an effect on total body weight, since increases in lean body mass and decreases in fat may lead to small or minimal changes in total weight.

## EFFECT OF CHROMIUM SUPPLEMENTATION ON SERUM LIPIDS

The effect of chromium to alter lipid profiles has been part of the many studies reporting effects on carbohydrate metabolism. Variable effects on one or more components of the serum lipid profile have been reported in studies of patients with diabetes or glucose intolerance as well as those from normal subjects [14–16, 19, 23, 30, 34–36, 38–41, 51, 52, 70–74].

## CHROMIUM SUPPLEMENTATION AND CARDIOVASCULAR DISEASE RISK

There has been great interest in evaluating the effect of chromium intake on CVD. However, the goal has been hampered by the fact that reliable blood levels to assess chromium are not readily available. In addition, the development of CVD is a process occurring over many years. In order to adequately evaluate the effect of chromium on this process, frequent blood testing is required. Due to these concerns and study

limitations, there has been interest in assessing tissue levels that may be more reflective of long-term chromium status. As such, use of toenail chromium has been suggested as one tool for such use.

Two epidemiologic studies have evaluated the relationship between Cr levels in toenails (a measure proposed that best reflects long-term intake of trace elements) and risk of coronary heart disease in men. The Health Professionals' Follow-up Study (HPFS) evaluated 33,737 male health care professionals in the US who were free of chronic disease and who provided toenail samples in 1987. They were followed prospectively for 7 years. It was reported in this study that there were 367 confirmed myocardial infarctions (MIs). Two control subjects were matched to each case subject. The risk for MI was reported to be significantly reduced in men in the highest quintile for toenail $Cr^{3+}$. However, this relationship was only significant for subjects with BMI $\geq$ 25 kg/m$^2$ [75]. In a second study conducted by the HPFS [76], mean toenail chromium (microgram per gram) was 0.71 in healthy control subjects ($n = 361$), 0.61 in diabetic subjects ($n = 688$), and 0.52 in diabetic men with prevalent CVD ($n = 198$, $P = 0.003$ for trend). In the cross-sectional analysis, after adjustment of potential confounders, the odds ratio (OR) between extreme quartiles was 0.74 (95% CI 0.49–1.11; $P = 0.18$ for trend) comparing diabetic with healthy control subjects. A similar comparison between diabetic men with prevalent CVD and healthy control subjects yielded an OR of 0.45 (95% CI 0.24–0.84; $P = 0.003$ for trend). A nested case-control analysis comparing diabetic men with incident CVD with healthy individuals yielded similar results. The authors of these reports suggested that adequate chromium may be important for both diabetes and CVD prevention.

The observations reported for the HPFS cohort appear to be consistent with observations reported from the European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer (EURAMIC). The EURAMIC was an incident, population-based, case-control study conducted in eight European countries and Israel to determine whether low toenail chromium concentrations are significantly associated with increased risk for MI. The study included 684 case subjects (men with a first diagnosis of MI within 24 hours of admission to the hospital) and 724 control subjects (men with similar demographic characteristics, but without MI). Average toenail chromium was 1.10 mg/kg in the case subjects versus 1.30 mg/kg in the control subjects. Additional analysis indicated that the adjusted ORs for MI for chromium quintiles 1–5 were 1.00, 0.82, 0.68, 0.60, and 0.59, respectively [77]. The results of EURAMIC thus indicate that toenail chromium concentration has a clearly inverse relationship with MI risk in men. This relationship remained significant after adjusting for age, BMI, HDL cholesterol, diabetes, history of hypertension, and smoking.

## CHROMIUM SUPPLEMENTATION AND DEPRESSION

There has been interest in assessing the effect of chromium on other disease states, particularly, psychiatric conditions such as depression. Depression has been associated with insulin resistance [78] and it is conceivable that increased insulin sensitivity leads to enhanced central noradrenergic and serotonergic activity. Postsynaptic brain serotonin receptor down-regulation by chromium in humans has also been reported which could

relate to insulin sensitivity and depression [79]. A small, double-blind, randomized, and placebo-controlled pilot study in 15 patients with major depression suggested that Cr may be effective in the treatment of atypical depression. Seventy percent of the subjects responded to Cr with no negative side effects [80].

In a second, double-blind, multicenter, 8-week replication study, 113 adult outpatients with atypical depression were randomized to receive 600 μg/day of elemental chromium in the ratio of 2 : 1, as provided by chromium picolinate (CrPic), or placebo. Primary efficacy measures were the 29-item Hamilton Depression Rating Scale (HAM-D-29) and the Clinical Global Impressions Improvement Scale (CGI-I). The results of this study suggested that the main effect of chromium was on carbohydrate craving and appetite regulation in depressed patients and that 600 μg of elemental chromium may be beneficial for patients with atypical depression who also have severe carbohydrate craving [81].

## SUMMARY

Chromium supplementation is widely practiced by individuals both with and without chronic diseases such as diabetes. The data reported from chromium supplementation studies in the past have yielded conflicting results. The reason why chromium supplementation was ineffective in some studies is not clear, but it is worth noting that the trials that demonstrated negative effects reported using relatively low chromium doses ($\leq 250$ μg/day), different forms of chromium, or evaluated study populations composed of both diabetic and nondiabetic patients. It does appear, however, that results from the trials using higher dose of chromium supplementation, especially in the form of CrPic type 1, appear to have favorable effects on parameters assessing carbohydrate metabolism. In addition, several large epidemiologic studies have suggested a relationship with chromium status and CVD risk. The fact these studies evaluated a maker suggestive of long-term chromium status, that is toenail chromium, is of interest given the fact that atherosclerosis is a process that develops over time.

With the current understanding of chromium's actions and the availability of more precise assessments for both CVD disease and insulin action, the next few years hold considerable promise in clarifying the clinical role of chromium supplementation.

## REFERENCES

[1] Riddle, M. C. (2005) Glycemic Management of type 2 diabetes: An emerging strategy with oral agents, insulins and combinations. *Endocrinol. Metab. Clin. North. Am. 34*, 77–98.

[2] Gibson, J. E., and Taylor, D. A. (2005) Can Claims, misleading information and manufacturing issues regulating dietary supplements be improved in the United States of America? *J. Pharmacol. Exp. Ther. 314*, 939–944.

[3] Neuhouser, M. L. (2003) Dietary supplement use by Amercian women: Challenges in assessing patterns of use, motives and costs. *J. Nutr. 133*, 1992S–1996S.

[4] Cefalu, W. T, and Hu, F. B. (2004) Role of chromium in human health and in diabetes. *Diabetes Care 27*, 2742–2751.

[5] Schwarz, K., and Mertz, W. (1959) Chromium(III) and the glucose tolerance factor. *Arch. Biochem. Biophys. 85*, 292–295.

[6] Tuman, R. W., and Doisy, R. J. (1977) Metabolic effects of the glucose tolerance factor (GTF) in normal and genetically diabetic mice. *Diabetes 26*, 820–826.
[7] Jeejeebhoy, K. N., Chu, R. C., Marliss, E. B., Greenberg, G. R., and Bruce-Robertson, A. (1977) Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parental nutrition. *Am. J. Clin. Nutr. 30*, 531–538.
[8] Freund, H., Atamian. S., and Fischer, J. E. (1979) Chromium deficiency during total parenteral nutrition. *JAMA 241*, 496–498.
[9] Anderson, R. A. (1995) Chromium and parenteral nutrition. *Nutrition 11*, 83–86.
[10] Kimura, K. (1986) Role of essential trace elements in the disturbance of carbohydrate metabolism. *Nippon Rinsho 54*, 79–84.
[11] Anderson, R. A. (1997) Nutritional factors influencing the glucose/insulin system: Chromium. *J. Am. Coll. Nutr. 16*, 404–410.
[12] American Diabetes Association. (2006) Standards of medical care in diabetes-2006. *Diabetes Care 29*, S4–42.
[13] Potter, J. F., Levin, P., Anderson. R. A., Freiberg, J. M., Andres, R., and Elahi, D. (1985) Glucose metabolism in glucose-intolerant older people during chromium supplementation. *Metabolism 34*, 199–204.
[14] Uusitupa, M. I., Mykkanen, L., Siitonen, O., Laakso, M., Sarlund, H., Kolehmainen, P., Rassanen, T., Kumpulainen, J., and Pyorälä, K. (1992) Chromium supplementation in impaired glucose tolerance of elderly: Effects on blood glucose, plasma insulin, C-peptide and lipid levels. *Br. J. Nutr. 68*, 209–216.
[15] Wilson, B. E., and Gondy, A. (1995) Effects of chromium supplementation on fasting insulin levels and lipid parameters in healthy, non-obese young subjects. *Diabetes. Res. Clin. Prac. 28*, 179–184.
[16] Rabinowitz, M. B., Gonick, H. C., Levin, S. R., and Davidson, M. B. (1983) Effects of chromium and yeast supplements on carbohydrate and lipid metabolism in diabetic men. *Diabetes Care 6*, 319–327.
[17] Trow, L. G., Lewis, J., Greenwood. R. H., Sampson, M. J., Self, K. A., Crews, H. M., and Fairweather-Tait, S. J. (2000) Lack of effect of dietary chromium supplementation on glucose tolerance, plasma insulin and lipoprotein levels in patients with type 2 diabetes. *Int. J. Vitam. Nutr. Res. 70*, 14–18.
[18] Sherman, L., Glennon, J. A., Brech, W. J., Klomberg, G. H., and Gordon, E. S. (1968) Failure of trivalent chromium to improve hyperglycemia in diabetes mellitus. *Metabolism 17*, 439–442.
[19] Uusitupa, M. I., Kumpulainen, J. T., Voutilainen, E., Hersio, K., Sarlund, H., Pyorala, K. P., Koivistoinen, P. E., and Lehto, J. T. (1983) Effect of inorganic chromium supplementation on glucose tolerance, insulin response, and serum lipids in noninsulin-dependent diabetics. *Am. J. Clin. Nutr. 38*, 404–410.
[20] Mossop, R. T. (1983) Effects of chromium(III) on fasting glucose, cholesterol and cholesterol HDL levels in diabetics. *Cent. Afr. J. Med. 29*, 80–82.
[21] Nath, R., Minocha, J., Lyall, V., Sunder, S., Kumar, V., Kapoor, S., and Dhar, K. L. (1979) Assessment of chromium metabolism in maturity onset and juvenile diabetes using chromium 51 and therapeutic response of chromium administration on plasma lipids, glucose tolerance and insulin levels in *Chromium in Nutrition and Metabolism* (Shapcott, D., and Hubert, J., eds) pp. 213–222, Elsevier/North Holland, Amsterdam.
[22] Glinsmann, W. H., and Mertz, W. (1966) Effect of trivalent chromium on glucose tolerance. *Metabolism 15*, 510–520.
[23] Amato, P., Morales, A. J., and Yen, S. S. (2000) Effects of chromium picolinate supplementation on insulin sensitivity, serum lipids, and body composition in healthy, nonobese, older men and women. *J. Gerontol. A Biol. Sci. Med. Sci. 55*, M260–M263.

[24] Jovanovic, L., Gutierrez, M., and Peterson, C. M. (1999) Chromium supplementation for women with gestational diabetes mellitus. *J. Trace Elem. Exp. Med. 12*, 91–97.
[25] Ravina, A., Slezak. L., Mirsky, N., Bryden, N. A., and Anderson, R. A.. (1999) Reversal of corticosteroid-induced diabetes mellitus with supplemental chromium. *Diabet. Med. 16*, 164–167.
[26] Morris, B. W., Kouta, S., Robinson, R., MacNeil, S., and Heller, S. (2000) Chromium supplementation improves insulin resistance in patients with type 2 diabetes mellitus. *Diabet. Med. 17*, 684–685.
[27] Cheng, N., Zhu, X., Shi, H., Wu, W., Chi, J., Cheng, J., and Anderson, R. (1999) Follow-up survey of people in China with type 2 diabetes mellitus consuming supplemental chromium. *J. Trace Elem. Exp. Med. 12*, 55–60.
[28] Anderson, R. A. (1998) Chromium, glucose intolerance and diabetes. *J. Am. Coll. Nutr. 17*, 548–555.
[29] Morris, B. W., MacNeil, S., Stanley, K., Gray, T. A., and Fraser, R. (1993) The interrelationship between insulin and chromium in hyperinsulinaemic euglycaemic clamps in healthy volunteers. *J. Endocrinol. 139*, 339–345.
[30] Cefalu, W. T., Bell-Farrow, A. D., Stegner, J., Wang, Z. Q., King, T., Morgan, T., and Terry, J. G. (1999) Effect of chromium picolinate on insulin sensitivity in vivo. *J. Trace Elem. Exp. Med. 12*, 71–83.
[31] Rossetti, L., Giaccari, A., and DeFronzo, R. A. (1990) Glucose toxicity. *Diabetes Care 13*, 610–630, 1990.
[32] Inzucchi, S. E., Maggs, D. G., Spollett, G. R., Page, S. L., Rife, F. S., Walton, V., and Shulman, G. I. (1998) Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N. Engl. J. Med. 338*, 867–872.
[33] Cefalu, W. T., Bell-Farrow, A. D., Wang, Z. Q., McBride, D., Dalgleish, D., and Terry, J. G. (1998) Effect of glipizide GITS on insulin sensitivity, glycemic indices, and abdominal fat composition in NIDDM. *Drug Dev. Res. 44*, 1–7.
[34] Anderson, R. A., Cheng, N., Bryden, N. A., Polansky, M. M., Chi, J., and Feng, J. (1997) Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.
[35] Ghosh, D., Bhattacharya, B., Mukherjee, B., Manna, B., Sinha, M., Chowdhury, J., and Chowdhury, S. (2002) Role of chromium supplementation in Indians with type 2 diabetes mellitus. *J. Nutr. Biochem. 13*, 690–697.
[36] Ravina, A., Slezak, L., Rubal, A., and Mirsky, N. (1995) Clinical use of the trace element chromium (III) in the treatment of diabetes mellitus. *J. Trace Elem. Exp. Med. 8*, 183–190.
[37] Bahijri, S. M., and Mufti, A. M. (2002) Beneficial effects of chromium in people with type 2 diabetes, and urinary chromium response to glucose load as a possible indicator of status. *Biol. Trace Elem. Res. 85*, 97–109.
[38] Bahijri, S. M., Mira, S. A., Mufti, A. M., and Ajabnoor, M. A. (2000) The effects of inorganic chromium and brewer's yeast supplementation on glucose tolerance, serum lipids and drug dosage in individuals with type 2 diabetes. *Saudi Med. J. 21*, 831–837.
[39] Chen, S. H., Sun, Y. P., and Chen, X. S. (1997) Effect of jiangtangkang on blood glucose, sensitivity of insulin and blood viscosity in non-insulin dependent diabetes mellitus [in Chinese]. *Zhongguo Zhong Xi Yi Jie He Za Zhi 17*, 666–668.
[40] Abraham, A. S., Brooks, B. A., and Eylath, U. (1992) The effects of chromium supplementation on serum glucose and lipids in patients with and without non-insulin-dependent diabetes. *Metabolism 41*, 768–771.
[41] Lee, N. A., and Reasner, C. A. (1994) Beneficial effect of chromium supplementation on serum triglyceride levels in NIDDM. *Diabetes Care 17*, 1449–1452.
[42] DeFronzo, R. A.. (1992). Insulin resistance, hyperinsulinemia, and coronary artery disease: A complex metabolic web. *J. Cardiovasc. Pharmacol. 20* (Supplement 11), S1–S16.

[43] Reaven, G. M. (1988) Banting lecture 1988: Role of insulin resistance in human disease. *Diabetes 37*, 1595–1607.
[44] Haffner, S. M. (1996) The insulin resistance syndrome revisited. *Diabetes Care 19*, 275–277.
[45] Liese, A. D., Mayer-Davis, E. J., and Haffner, S. M. (1998) Development of the multiple metabolic syndrome: An epidemiologic perspective. *Epidemiol. Rev. 20*, 157–172.
[46] Isomaa, B., Almgren, P., Tuomi, T., Forsén, B., Lahti, K., Nissén, M., Taskinen, M-R., and Groop, L. (2001) Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care 24*, 683–689.
[47] The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol in Adults (2001) Executive Summary of the Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) *JAMA 285*, 2486–2497.
[48] Hunter, S. J., and Garvey, W. T. (1998) Insulin action and insulin resistance: Diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. *Am. J. Med. 105*, 331–345.
[49] Gunton, J. E., Cheung, N. W., Hitchman, R., Hams, G., O'Sullivan, C., Foster-Powell, K., and McElduff, A. (2005) Chromium supplementation does not improve glucose tolerance, insulin sensitivity, or lipid profile: A randomized, placebo-controlled, double-blind trial of supplementation in subjects with impaired glucose tolerance. *Diabetes Care 28*, 712–713.
[50] Komorowski, J., and Juturu, V. (2005) Chromium supplementation does not improve glucose tolerance, insulin sensitivity, or lipid profile: A randomized, placebo-controlled, double-blind trial of supplementation in subjects with impaired glucose tolerance. *Diabetes Care 28*, 1841–1842.
[51] Volpe, S. L., Huang, H. W., Larpadisorn, K., and Lesser, I. I. (2001) Effect of chromium supplementation and exercise on body composition, resting metabolic rate and selected biochemical parameters in moderately obese women following an exercise program. *J. Am. Coll. Nutr. 20*, 293–306.
[52] Offenbacher, E. G., and Pi-Sunyer, F. X. (1980) Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects. *Diabetes 29*, 919–925.
[53] Althuis, M. D., Jordan, N. E., Ludington, E. A., and Wittes, J. T. (2002) Glucose and insulin responses to dietary chromium supplements: A meta-analysis. *Am. J. Clin. Nutr. 76*, 148–155.
[54] Anderson, R. A. (2003) Chromium and insulin sensitivity. *Nutr. Res. Rev. 16*, 267–275.
[55] Juturu, V., and Komorowski, J. R. (2003) Chromium supplements, glucose, and insulin responses. *Am. J. Clin. Nutr. 78*, 190–193.
[56] Cefalu, W. T., Wang, Z. Q., Zhang, X. H., Baldor, L. C., and Russell, J. C. (2002) Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle glut-4 translocation in obese, hyperinsulinemic (JCR-LA Corpulent) Rats. *J. Nutr. 132*, 1107–1114.
[57] Wang, Z. Q., Zhang, X. H., Russell, J. C., Hulver, M., and Cefalu, W. T. (2006) Chromium picolinate enhances skeletal muscle cellular insulin signaling in vivo in obese, insulin resistant JCR:LA-cp rat. *J. Nutr.*, *136*, 415–420.
[58] Centers for Disease Control and Prevention. (CDC) (2003) Diabetes prevalence among American Indians and Alaska Natives and the overall population—United States, 1994–2002. *MMWR Morb. Mortal Wkly. Rep. 52*, 702–704.
[59] Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S., and Marks, J. S. (2003) Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA 289*, 76–79.

[60] Ford, E. S., Giles, W. H., and Dietz, W. H. (2002) Prevalence of the metabolic syndrome among US adults: Findings from the third National Health and Nutrition Examination Survey. *JAMA 287*, 356–359.

[61] *Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation*. WHO Technical Report Series, 894 (2000) World Health Organization, Geneva, Switzerland.

[62] Evans, R. M., Barish, G. D., and Wang, Y. U. (2004) PPARs and the complex journey to obesity. *Nat. Med. 10*, 355–361.

[63] Crawford, V., Scheckenbach, R., and Preuss, H. G. (1999) Effects of niacin-bound chromium supplementation on body composition in overweight African-American women. *Diabetes Obes. Metab. 1*, 331–337.

[64] Campbell, W. W., Joseph, L. J., Anderson, R. A., Davey, S. L., Hinton, J., and Evans, W. J. (2002) Effects of resistive training and chromium picolinate on body composition and skeletal muscle size in older women. *Int. J. Sport Nutr. Exerc. Metab. 12*, 125–135.

[65] Lukaski, H. C., Bolonchuk, W. W., Siders, W. A., and Milne, D. B. (1996) Chromium supplementation and resistance training: effects on body composition, strength, and trace element status of men. *Am. J. Clin. Nutr. 63*, 954–965.

[66] Hallmark, M. A., Reynolds, T. H., DeSouza, C. A., Dotson, C. O., Anderson, R. A., and Rogers, M. A.. (1996) Effects of chromium and resistive training on muscle strength and body composition. *Med. Sci. Sports Exerc. 28*, 139–144.

[67] Kaats, G. R., Blum, K., Fisher, J.A., and Adelman, J. A. (1996) Effects of chromium picolinate supplementation on body composition: a randomized, double-masked placebo-controlled study. *Curr. Ther. Res. 57*, 747–756.

[68] Kaats, G. R., Blum, K., Pullin, D., Keith, S. C., and Woods, R. (1998) A randomized, double-masked, placebo-controlled study of the effects of chromium picolinate supplementation on body composition: A replication and extension of a previous study. *Curr. Ther. Res. 59*, 379–388.

[69] Pittler, M. H., Stevinson, C., and Ernst, E. (2003) Chromium picolinate for reducing body weight: Meta-analysis of randomized trials. *Int. J. Obes. Relat Metab Disord. 27*, 522–529.

[70] Roeback, J. R., Jr, Hla, K. M., Chambless, L. E., and Fletcher, R. H. (1991) Effects of chromium supplementation on serum high-density lipoprotein cholesterol levels in men taking beta-blockers: A randomized, controlled trial. *Ann. Intern. Med. 115*, 917–924.

[71] Evans, G. W. (1989) The effect of chromium picolinate on insulin controlled parameters in humans. *Int. J. Biosoc. Med. Res. 11*, 163–180.

[72] Riales, R., and Albrink, M. J. (1981) Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high-density lipoprotein of adult men. *Am. J. Clin. Nutr. 34*, 2670–2678.

[73] Press, R. I., Geller, J., and Evans, G. W. (2000) The effect of chromium picolinate on serum cholesterol and apolipoprotein fractions in human subjects. *West. J. Med. 152*, 41–45.

[74] Preuss, H. G., Wallerstedt. D., and Talpur, N. (2000) Effects of niacin-bound chromium and grape seed proanthocyanidin extract on the lipid profile of hypercholesterolemic subjects: A pilot study. *J. Med. 31*, 227–246.

[75] Rimm, E. B., Guallar, E., Giovannucci, E., Ascherio, A., Stampfer, M. J., Willet, W. C., and Hu, F. (2002) Toenail chromium levels and risk of coronary heart disease among normal and overweight men. Presented at the *American Heart Association 42nd Annual Conference on Cardiovascular Disease, Epidemiology and Prevention, 23–26 April 2002, Honolulu, Hawaii.*

[76] Rajpathak, S., Rimm, E. B., Li, T., Morris, J. S., Stampfer, M. J., Willett, W. C., and Hu, F. B. (2004) Lower toenail chromium in men with diabetes and cardiovascular disease compared with healthy men. *Diabetes Care 27*, 2211–2216.

[77] Guallar, E., Jimenez, F. J., van't Veer, P., Bode, P., Riemersma, R. A., Gomez-Aracena, J., Kark, J. D., Arab, L., Kok, F. J., and Martin-Moreno, J. M. (2005) EURAMIC-Heavy Metals and Myocardial Infraction Study Group. Low toenail chromium concentration and increased risk of nonfatal myocardial infarction. *Am. J. Epidemiol. 162*, 157–164.

[78] Paykel, E. S., Mueller, P. S., and De la Vergne, P. M. (1973) Amitriptyline, weight gain and carbohydrate craving: a side effect. *Br. J. Psychiatry 123*, 501–507.

[79] Attenburrow, M. J., Odontiadis, J., Murray, B. J., Cowen, P. J., and Franklin, M. (2002) Chromium treatment decreases the sensitivity of 5-HT2A receptors. *Psychopharmacology (Berl) 159*, 432–436.

[80] Davidson, J. R., Abraham, K., Connor, K. M., and McLeod, M. N. (2003) Effectiveness of chromium in atypical depression: a placebo-controlled trial. *Biol. Psychiatry 5*, 261–264.

[81] Docherty, J. P., Sack, D.A., Roffman, M., Finch, M., and Komorowski, J. R. (2005) A double-blind, placebo-controlled, exploratory trial of chromium picolinate in atypical depression: effect on carbohydrate craving. *J. Psychiatr. Pract. 11*, 302–314.

[82] Martin, J., Wang, Z. Q., Zhang, X. H., Wachtel, D., Volaufova, J., Matthews. D. E., and Cefalu, W. T. (2006) Chromium picolinate supplementation attenuates body weight gain and increase insulin sensitivity with type 2 diabetes. *Diabetes Care 29*, 1826–1832.

[83] Kleefstra, N., Houweling. S. T., Jansman, F. G. A., Groenier, K. H., Gans, R. O . B., Meyboom-de Jong, B., Bakker, S. J. L., and Bilo, H. J. G. Chromium treatment has no effect in patients with poorly controlled, insulin-treated type 2 diabetes in an obese Western population: a randomized, double-blind, placebo-controlled trial. *Diabetes Care 29,* 521–525, 2006.

This page intentionally left blank

*Chapter 9*

# Benefits of chromium(III) complexes in animal and human health

**S. Zafra-Stone,[1] M. Bagchi,[1] H. G. Preuss,[2] and D. Bagchi[1,3,*]**

[1]InterHealth Research Center, Benicia, CA 94510; [2]Georgetown University Medical Center, Washington, DC 20007; and [3]Department of Pharmacy Sciences, Creighton University Medical Center, Omaha, NE 68178

## INTRODUCTION

Chromium, one of the most common elements in the earth's crust and sea water, exists mainly in five valence states: metallic chromium [Cr(0)], trivalent chromium [Cr(III)], bivalent chromium [Cr(II)], pentavalent chromium [Cr(V)], and hexavalent chromium [Cr(VI)] [1]. Chromium(VI) is prepared by oxidizing naturally occurring Cr(III) and used extensively in industrial chemicals including steel, chrome plating, welding, painting, metal finishes, steel manufacturing, alloy, cast iron, and wood treatment. Chromium(VI) is widely known to cause allergic dermatitis as well as toxic and carcinogenic effects in animals and humans. Although chromium(V) has been identified as the ultimate carcinogen, investigators have demonstrated that Cr(V) complexes, produced in the reduction of Cr(VI) by cellular reductants, generate hydroxyl radicals and cause DNA damage. Chromium(III) is relatively non-toxic and identified as a novel micronutrient for its beneficial role in human nutrition by serving as a critical cofactor in the action of insulin as well as nutritional enhancement to energy, glucose, and lipid metabolism [2]. However, Ozawa et al. in 1990 demonstrated that Cr(III) can be reduced to Cr(II) by the biological reductants L-cysteine and NADH, and the newly formed Cr(II) reacts with hydrogen peroxide to generate hydroxyl radicals, which can be detected by erthrocyte sedimentation rate(ESR) and high performance liquid chromatography(HPLC) [3].

## CHROMIUM DEFICIENCY AND DISEASES

Chromium deficiency is a wide spread problem. Many people such as athletes, diabetics, pregnant women, and the elderly are especially at risk of chromium deficiency leading

[*] The invited author (and primary author) of this chapter.

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

to impaired insulin function, inhibition of protein synthesis and energy production, and to type 2 diabetes and heart disease [4]. Numerous studies have shown that a strong association exists between chromium deficiency, high blood insulin, and elevated blood cholesterol levels. In rats, chromium deficiency has been shown to increase serum cholesterol levels and formation of aortic plaques [4]. However, adding chromium to the diet prevented both the formation of aortic plaques and the rise of serum cholesterol [2].

Further, chromium losses are found in diets with large quantities of refined foods, especially simple sugars, which exacerbates the problem since these foods are not only low in chromium but also increase losses of chromium through the urine. Several experiments in animals have also demonstrated that high sucrose, chromium deficient diets potentially cause severe atherosclerosis. Additional forms of chromium loss are found during long periods of stress such as pregnancy, infection, physical trauma, and strenuous exercise. Exercise has been shown to induce chromium loss in athletes and lead to chromium deficiency resulting in impaired insulin function [5].

## DIETARY SOURCES OF BENEFICIAL CHROMIUM

Significant dietary sources of trivalent chromium are available in various food sources such as whole-grain products, high-bran breakfast cereals, egg yolks, coffee, nuts, green beans, broccoli, meat, Brewers' yeast, and selected brands of beer and wine. Chromium is also found in many mineral or multivitamin supplements. According to the National Research Council (NRC), the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for trivalent chromium is 50–200 μg/day, corresponding to 0.83–3.33 μg/kg/day for an adult weighing 60 kg [6]. The Food and Drug Administration (FDA) has selected a Reference Daily Intake (RDI) of 120 μg/day for chromium. However, normal dietary intake of chromium for adults may be less than optimal. Recent reports demonstrate that the normal US adult chromium dietary intake is less than 50 μg/day, resulting in approximately 0.4% chromium absorption. Results from one study indicated that daily chromium intakes for men and women in the US were 33 and 25 μg, respectively [7]. Fortunately, trivalent chromium significantly influences bioavailability. At a dose of 1000 μg/day absorption of chromium from chromium chloride ($CrCl_3$) is ~0.4%, whereas that from chromium picolinate may be as high as 2.8%. Once absorbed, chromium is distributed widely in the body, with the highest levels being found in the kidney, liver, spleen, and bone [8].

## TRIVALENT CHROMIUM, ABSORPTION, AND BIOAVAILABILITY

Several Cr(III) organic complexes have shown to have significantly high absorption potential and tissue incorporation activity. Nutritional research on trivalent chromium has primarily focused on Cr(III) complexes with organic acids, including nicotinic acid or niacin and picolinic acid as well as chromium amino acid chelates. Current methods used for assaying chromium by atomic absorption spectrometry utilize graphite furnace or neutron activation, which are sufficiently sensitive and specific to evaluate chromium levels in blood, urine, or hair [9].

The mechanism of chromium absorption and action has been recently revealed on a molecular level. After absorption in the gastrointestinal tract, chromium is transported to cells bound to the plasma protein transferrin. Insulin initiates chromium transport into the cells where it is bound to the oligopeptide apochromodulin. Apochromodulin, combined in a tetra-nuclear assembly of four Cr(III) atoms, forms the low-molecular weight oligopeptide chromodulin (MW $\sim$ 1500 Da) which is important in amplifying the insulin signaling effect. After binding to insulin-activated receptor, chromodulin increases tyrosine kinase activity and forms a part of intracellular portion of insulin receptor [9]. In a recent study conducted by Clodfelder et al. in 2004, the biomimetic cation $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ was found to imitate the oligopeptide chromodulin's ability to stimulate the tyrosine kinase activity of insulin receptor, increase insulin sensitivity, decrease plasma total concentrations, low-density lipoprotein cholesterol, and triglycerides concentrations as shown in healthy and type 2 diabetic rat models. In addition, due to the stability and solubility of the biomimetic cation, a greater magnitude of absorbability is demonstrated in comparison to marketed nutritional supplements such as $CrCl_3$, niacin-bound chromium (NBC), or chromium picolinate. At nutritional supplement and pharmacological levels, $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ is efficiently absorbed at greater than 60 and 40%, respectively [10].

However, no matter the form of chromium used, the ability to absorb or convert inorganic chromium to the active form appears to decrease with advancing age. Studies demonstrated that the normal adult response time to inorganic chromium treatment requires 1–3 months; whereas in children response time to inorganic chromium was typically within 24 hours [11]. As the human body ages, tissue chromium levels decrease and urinary excretion of chromium increases, elevating the risk of heart disease, hypertension and diabetes, increased blood cholesterol, and blood pressure [9, 12, 13].

In a study conducted by Feng et al. in 1998, insulin-dependent rats were shown to absorb and excrete more chromium than the normal rats using actively enriched stable isotope Cr-50 compound $Cr_2O_3$ as a tracer [14]. The comparison of absorption, distribution, and excretion in organs and tissues of the two groups demonstrated some alteration; however, some differences indeed exist. The contents of $^{51}Cr$ radioactivity of the diabetic rats appear to be of higher retention than in most studied organisms. Urinary excretion of $^{51}Cr$ in diabetics was found to be significantly higher than that of normal rats. Overall, results demonstrated that absorption of chromium is dependent on the insulin sensitivity [14].

Clinical studies have shown that diabetic patients have lower serum chromium levels and a higher chromium excretion rate. In a study conducted by Clodfelder et al. in 2004, the effects of type 1 and type 2 diabetes on the transport of chromium included greater urinary chromium loss, and greater movement of chromium from the blood to the tissues, most notably to the skeletal muscle; and an alteration of the distribution of chromium in the blood plasma was found in diabetic rat models compared to healthy rats [15].

In better defining the metabolism of trivalent chromium complexes with respect to both its absorption and cellular action, Olin et al. in 1994 investigated the absorption and retention of $CrCl_3$, niacin-bound chromium, chromium picolinate over a 12-hour period in a rat model [16]. Male rats (150–170 g) were gavaged with 44 $\mu$Ci (1 mL each or 2.7 nmol) chromium as $CrCl_3$, niacin-bound chromium, or chromium picolinate. At 1-, 3-, 6-, and 12-hour post-gavage, rats were anesthetized and killed. Cardiac blood and urine (at 6 and 12 hour) were collected, and liver, kidneys, pancreas,

testes, and gastrocnemius were removed, weighed, and assayed to calculate chromium absorbed/retained. Results showed the highest percent of absorbed/retained counts was in urine, then muscle, blood, and liver. The average percent chromium retained was higher in niacin-bound chromium-gavaged rats than in $CrCl_3$- or chromium picolinate-gavaged rats in the majority of the time points and tissues. One-hour post-gavage, niacin-bound chromium rats had retention percentages 3.2-fold to 8.4-fold higher in tissues collected compared to chromium picolinate and $CrCl_3$ groups. Three-hour post-gavage, niacin-bound chromium-treated rats had blood, muscle, and pancreatic chromium retentions that were 2.4–8 times higher compared to chromium picolinate-gavaged rats. By 6- and 12-hour post-gavage, niacin-bound chromium rats had absorbed/retained Cr levels in tissues that were 1.8 to 3.8 times higher than those of chromium picolinate rats. Results demonstrated significant differences in the bioavailability of the different chromium compounds (Figs 1–3) [16].

## CHROMIUM(III) AND GLUCOSE TOLERANCE FACTOR (GTF)

Chromium(III), in the form of the naturally occurring dinicotinic acid–glutathione complex, or glucose tolerance factor (GTF), significantly and strongly increases the effect of exogenous insulin on glucose metabolism. Glucose tolerance factor is an essential dietary component that potentiates the action of insulin and thereby functions in regulating protein, fat, and carbohydrate metabolism. Glucose tolerance factor differs from simple Cr(III) compounds due to absorbability, access to biologically important chromium storage deposits, placental absorption, and blood glucose stability. The most abundant and naturally occurring form of GTF is found in Brewer's yeast. The O-coordinated chromium(III)–dinicotinic acid complex is biologically active which suggests that a *trans* configuration of pyridine nitrogen atoms resembles that part of the GTF structure which is recognized by the receptors or enzymes which are involved in the expression of the biological effect. Glucose tolerance factor differs from simple chromium compounds

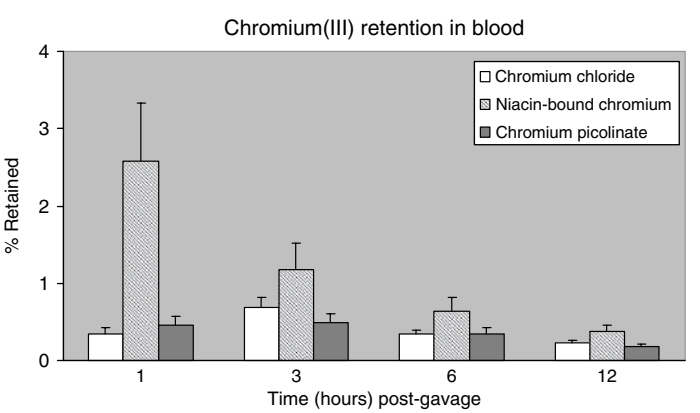



Fig. 1. Amount of $^{51}Cr$ absorbed/retained (% of dose) in the blood following supplementation of $CrCl_3$, niacin-bound chromium, and chromium picolinate.

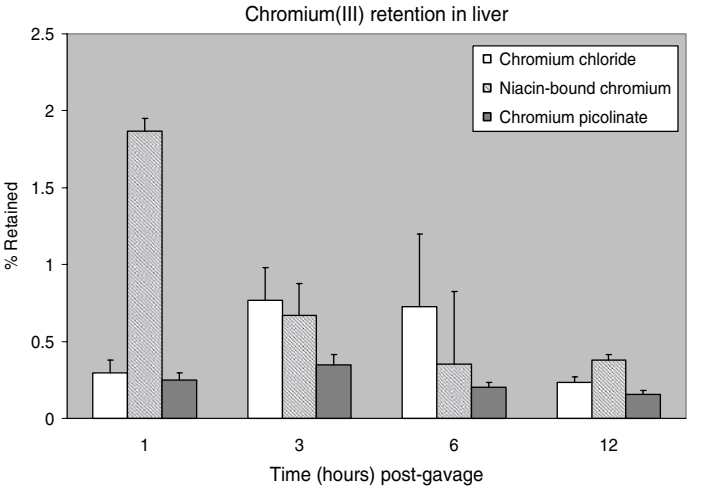



Fig. 2. Amount of $^{51}Cr$ absorbed/retained (% of dose) in the hepatic tissue following supplementation of $CrCl_3$, niacin-bound chromium, and chromium picolinate.

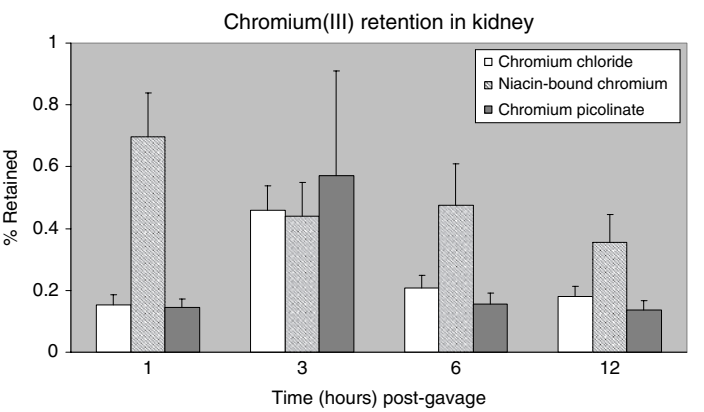



Fig. 3. Amount of $^{51}Cr$ absorbed/retained (% of dose) in the kidney tissue following supplementation of $CrCl_3$, niacin-bound chromium, and chromium picolinate.

in that it is easily absorbed, has access to biologically important storage deposits of chromium, access to fetus, stabilizes blood glucose, and is safe [17]. The body's ability to convert simple Cr(III) compounds into the GTF form declines with advancing age and is impaired in diabetes and probably in hyperlipidemic and atherosclerotic patients as well [18–20]. Unfortunately, naturally occurring GTF comprises less than 2% of the available chromium in Brewer's yeast. Dietary adequacy is thereby difficult.

Several studies demonstrated that the biologically active form of Cr(III) in Brewer's yeast promotes glucose tolerance and prevented diabetes in experimental animals by

impeding the action of insulin and enhancing protein, fat, and carbohydrate metabolism and significantly reduced plasma glucose levels in diabetic mice [18, 19]. A clinical investigation on diabetic patients showed a highly favorable response to chromium supplementation via Brewer's yeast and increased insulin sensitivity. Twenty-two participants (8 males and 14 females; mean age 51 years) with fasting values of total cholesterol and glucose from 3.21 to 6.90 and 4.3 to 6.2 mmol/L, respectively, were evaluated. After 9 hour fast, oral glucose load (75 g) was administered before chromium supplementation and blood was drawn before and at 30, 60, 90, and 120 minutes after the glucose load [20, 21]. Subjects were given either Brewer's yeast or Torula yeast (10 g yeast powder) daily for 12 weeks. Brewer's yeast demonstrated a beneficial effect of decreasing serum triacylglycerol values in subjects. In an oral glucose tolerance test, an increment at 0 minute and significant decreases at 60 and 90 minutes were shown. In subjects given Torula yeast, glucose values increased at both 0 and 30 minutes after glucose load and 12-week supplementation. Brewer's yeast and Torula yeast significantly altered glucose concentrations at 60 minutes after glucose dosage. Brewer's yeast had significant decreasing effects on insulin output at both 90 and 120 minutes after glucose load. Likewise, in subjects given Torula yeast, serum insulin contents decreased at 90 minutes. Brewer's yeast supplementation had beneficial effects both on serum triacylglycerol and on 60- and 90-minute glucose values of oral glucose tolerance test [20, 21].

A complex of Cr(III) and nicotinic acid was demonstrated to facilitate insulin binding as in the role of GTF found in Brewer's yeast. Urberg et al. in 1987 showed that humans' inability to respond to chromium supplementation resulted from suboptimal levels of dietary nicotinic acid to serve as a substrate for GTF synthesis [20]. In a controlled clinical trial, 16 healthy elderly volunteers were given 200 μg Cr(III), 100 mg nicotinic acid, or 200 μg Cr(III) + 100 mg nicotinic acid daily for 28 days and evaluated on days 0 and 28. Fasting glucose and glucose tolerance were unaffected by either Cr(III) or nicotinic acid alone. In contrast, the combined chromium–nicotinic acid supplement caused a 15% decrease in glucose (area integrated total; $p < 0.025$) and a 7% decrease in fasting glucose [20, 21].

## CHROMIUM(III), DIABETES, AND INSULIN RESPONSE

Dietary trivalent chromium has been shown to play an important role in type 2 diabetes mellitus, gestational diabetes, steroid-induced diabetes, and glucose tolerance by providing significant beneficial effects on the insulin system, often enhancing insulin sensitivity and overcoming glucose intolerance. The relationship between Cr(III) and its effect on diabetes was determined by Davis and Vincent in 1997 in which chromium has been shown to bind to insulin receptor, thus resulting in the increase of tyrosine protein kinase activity [22].

Numerous clinical researches using dietary Cr(III) demonstrated significant beneficial effects on the insulin system. Anderson et al. in 1997 noted that chromium supplementation may prove to be a useful means to prevent or treat type 2 diabetes mellitus [23]. Several studies demonstrated that animals fed a chromium-deficient diet developed the earliest stage of diabetes, high blood insulin levels, which was reversed by adding chromium-rich foods [15, 24]. Research on epididymal fat tissue from chromium-deficient rats further suggested that chromium action was dependent on insulin. The

primary function of chromium is to potentiate the effects of insulin and thereby enhance glucose, amino acid, and fat metabolism [25].

A number of trivalent chromium supplements, at different doses, administered to patients with normal glucose tolerance, diabetes, and gestational diabetes have shown effective results in glucose levels and insulin metabolism. Numerous studies on niacin-bound chromium demonstrated its efficacy on glucose and insulin sensitivity. In a randomized, double-blind, placebo-controlled trial, 15 healthy subjects (5 males, 10 females) were given ~2 mg niacin-bound chromium/day for 90 days. The placebo group consisted of 11 subjects (6 males, 5 females). In subjects with relatively high fasting insulin levels at the beginning of the trial (6 subjects, 56 pmol/l), there was a decrease in fasting insulin levels after chromium supplementation (38 pmol/l at 90 days) [26].

In subjects with inadequate Cr(III), long-term glucose control by niacin-bound chromium has been demonstrated to improve altered glucose and lipid metabolism. In a double-blind, clinical investigation, two groups of volunteers received either 300 μg elemental Cr(III) as niacin-bound chromium or a placebo daily for 3 months. Mean fasting glucose levels were lowered significantly in the niacin-bound chromium supplemented group, while glucose levels remained unchanged in the placebo group. Niacin-bound, chromium-supplemented group also experienced a decrease in mean triglycerides and glycosylated hemoglobin (Hb1Ac), a biomarker for long-term glucose control. In contrast, mean Hb1Ac increased in the placebo group [27].

Research on niacin-bound chromium in combination with exercise training resulted in a lowered insulin response to an oral glucose load. In a study conducted by Grant et al. [28], niacin-bound chromium supplementation (400 μg elemental chromium/day) was given to young, obese women with or without exercise training. Exercise training combined with niacin-bound chromium supplementation resulted in a lowered insulin response to an oral glucose load as well as significant weight loss [28]. While under identical clinical conditions using chromium picolinate, subjects experienced significant weight gain [28].

Another study on the effects of niacin-bound chromium on GTF activity in humans was determined in a double-blind, randomized clinical fashion. Nineteen male and female volunteers received 300 μg elemental chromium as niacin-bound chromium for 3 months. Fasting glucose values were significantly lowered in the niacin-bound chromium-supplemented volunteers, while no significant change was observed in the placebo group (Fig. 4). Mean triglyceride levels reduced from 112 to 108 mg/dL in niacin-bound chromium-supplemented group. Mean Hb1Ac levels also lowered from 8.42% to 8.10%, while Hb1Ac level increased in the placebo group. No adverse effects were observed [29].

A recent study demonstrated the molecular mechanism of Cr(III) chloride supplementation which increases insulin sensitivity and glycemic control in cultured U937 monocytes. Jain et al. in 2001 demonstrated that $CrCl_3$ inhibits the secretion of TNF-$\alpha$, a cytokine known to inhibit insulin action and sensitivity. U937 monocytes were cultured in a high glucose medium and treated with and without $CrCl_3$. $CrCl_3$ supplementation prevented the increase in TNF-$\alpha$ levels as well as oxidative stress caused by the high glucose levels in cultured U937 monocytic cells. Similarly, $CrCl_3$ prevented elevated TNF-$\alpha$ secretion and lipid peroxidation levels in $H_2O_2$-treated U937 cells [30].

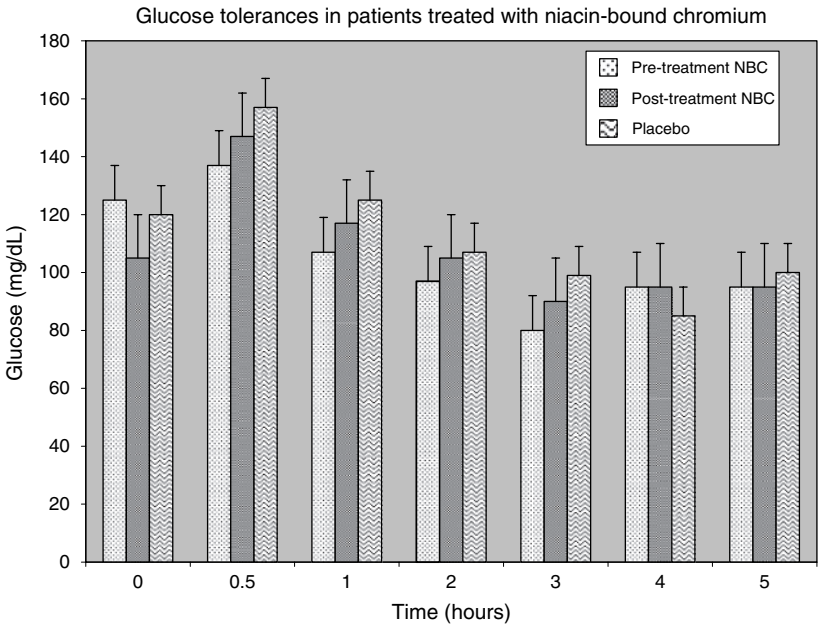



Fig. 4. Time-dependent effects of niacin-bound chromium supplementation on glucose tolerance in patients.

In a double-blind, 12-week study, 23 healthy adult men aged 31–60 years received either 200 μg elemental chromium as $CrCl_3$ in 5 mL water or 5 mL plain water daily 5 days each week. Half the subjects volunteered for glucose tolerance tests with insulin levels. Decreases in insulin and glucose were found in those subjects having normal glucose levels together with elevated insulin levels at baseline. Results suggested that $CrCl_3$ improved insulin sensitivity in subjects with evidence of insulin resistance but normal glucose tolerance [31].

In a similar study, $CrCl_3$ was shown to exhibit significant anti-diabetic potential in chemically induced diabetes in rats thus leading to improved peripheral insulin sensitivity. The effect of 6-week oral administration of $CrCl_3$ on the glucose and lipid metabolism was also studied in streptozotocin diabetic and neonatal-streptozotocin diabetic rats. Treatment with $CrCl_3$ significantly improved the impaired glucose tolerance and insulin sensitivity of both streptozotocin diabetic and neonatal-streptozotocin diabetic rats without any change in basal or glucose-stimulated insulin response indicating insulin-sensitizing action of chromium. $CrCl_3$ treatment also significantly improved deranged lipid metabolism [32].

Various researches on chromium picolinate and its effects on insulin sensitivity and mechanisms of anti-diabetic action were evaluated in various experimental models of diabetes mellitus. Chromium picolinate was shown to significantly decrease glucose of both type 1 and type 2 diabetic rats without any significant change as compared to controls. A significant increase in the composite insulin sensitivity index values of

both type 1 and type 2 diabetic rats were also determined. Results demonstrated that chromium picolinate significantly improves deranged carbohydrate and lipid metabolism of experimental, chemically induced diabetes in rats. According to Shindea et al. in 2004, the mechanism of in vivo anti-diabetic action appears to be the peripheral insulin enhancing action of chromium [33].

Cefalu et al. in 1999 indicated that chromium picolinate supplementation can improve insulin sensitivity in clinically obese and pre-diabetic individuals [34]. In a double-blind, placebo-controlled, clinical trial, 29 subjects at risk of developing type 2 diabetes were given either 1000 μg chromium picolinate per day or placebo for 8 months. The patients who received the chromium picolinate supplements showed a significant increase in insulin sensitivity at 4 and 8 months. Results were seen in the absence of significant changes in body fat distribution, demonstrating that chromium picolinate can beneficially affect insulin sensitivity independent of changes in weight or body fat percentage, therefore yielding a direct influence on muscle insulin action [34]. In a similar study conducted by Anderson et al. in 1997, 180 people being treated for type 2 diabetes supplemented with 200 or 1000 μg of chromium picolinate or placebo a day. Supplemental chromium demonstrated dramatic effects on glucose and insulin variables in individuals with type 2 diabetes. Significant and sustained reductions in diabetic symptoms were observed in subjects who received 1000 μg of chromium picolinate a day for 4 months [35]. A similar study by Evans, in 1989, in adult non-insulin-dependent diabetic patients demonstrated an average decrease of 32 mg/dL (or 18%) in blood sugar levels and 8 mg/dL or 8% decrease in low-density lipoprotein (LDL) following a daily administration of 200 μg of elemental chromium as chromium picolinate [36].

In another study on elderly diabetic patients, the effects of chromium picolinate on 39 patients within a rehabilitation program were investigated. Along with standard treatment for diabetes, the study group received 200 μg of elemental chromium twice a day for a 3-week period. Results demonstrated that dietary supplementation with chromium is beneficial in moderating glucose intolerance in elderly, diabetic patients undergoing rehabilitation [37].

Low-molecular-weight organic chromium complexes, such as chromium with phenylalanine, $Cr(pa)_3$, have shown to improve insulin responsiveness and reduce whole body glucose tolerance. This newly synthesized complex of Cr(III) chelated with D-phenylalanine ligand has shown to augment insulin-stimulated glucose-uptake in mouse 3T3-adipocytes. At the molecular level, $Cr(pa)_3$ was shown to enhance insulin-stimulated phosphorylation of Akt in a time- and concentration-dependent manner without altering the phosphorylation of insulin receptor. Oral treatment with $Cr(pa)_3$ (150 μg elemental Cr/kg body weight/day, for 6 weeks) in ob/ob+/+ obese mice significantly alleviated glucose tolerance compared with untreated obese mice [38].

## CHROMIUM(III) SUPPLEMENTATION IN LOWERING BLOOD PRESSURE, HYPERTENSION, AND CHOLESTEROL

According to the American Heart Association, nearly 100 million Americans have an average cholesterol level above 200 mg/dL or higher. Reports have shown that persons

with elevated insulin levels exhibit increased levels of LDL cholesterol, decreased high-density lipoprotein (HDL) cholesterol, and high blood pressure compared to individuals with normal insulin levels [12, 13]. Furthermore, as blood pressure increases steadily, especially in aging individuals, high blood pressure can lead to "essential hypertension" or "age-related hypertension" as well as "diabetic hypertension" all of which are associated with insulin perturbations [12, 13]. Many drugs lower circulating cholesterol levels, but they are not infrequently associated with severe side effects. Numerous studies have shown that Cr(III) can prevent hypertension by lowering harmful LDL cholesterol and increasing beneficial HDL cholesterol and even reverse atherosclerosis [12, 17]. Abraham et al. in 1991 reported that rabbits on a high cholesterol diet followed with chromium(III) chloride showed a significant regression of atherosclerosis plaques while rabbits without chromium showed no improvement [39].

Several animal studies have demonstrated that niacin and chromium exerted significant beneficial effects on serum lipid levels. In a study conducted by Bolkent et al. in 2004, 12-month-old female Swiss albino rats were examined to determine whether combined treatment with Cr(III) and niacin have beneficial effects in the hepatic tissues of animals fed a lipogenic diet. Four groups of animals were investigated: Group I (control) were fed standard rodent chow; Group II was orally treated with 250 μg/kg $CrCl_3$ and 100 mg/kg niacin for 45 days; Group III were fed a lipogenic diet consisting of 2% cholesterol, 0.5% cholic acid, and 20% sunflower oil added to the pellet chow and given 3% alcoholic water for 60 days; and Group IV was fed with the same lipogenic diet, and 15 days after the experimental animals were made hyperlipemic and orally treated with 250 μg/kg $CrCl_3.6H_2O$ and 100 mg/kg niacin for 45 days. On day 60, liver and blood samples were analyzed. Results demonstrated that a combination of chromium and niacin decreases total cholesterol and total lipid levels in serum significantly and niacin and Cr(III) regenerated some of the hepatocytes as well [40].

As discussed before, a strong association exists between Cr(III) deficiency, high blood insulin, and elevated blood cholesterol levels. While regular exercise and low fat, low cholesterol diets are integral part of a complete cholesterol lowering program, niacin-bound chromium supplementation may offer an effective method of combating high blood cholesterol [41, 42]. Lefavi et al. in 1993 studied the lipid-lowering effects of niacin-bound chromium administered to young athletic male adults for 8 weeks. In a randomized, placebo-controlled study, 34 male bodybuilders (age 18–28 years) were given either placebo, 200 μg NBC or 800 μg NBC daily for 8 weeks while training was controlled. Twelve-hour triglyceride, HDL cholesterol, HDL total cholesterol, LDL cholesterol, and one-hour post-challenge insulin, and glucose values were determined at pre- and post-supplementation periods. All pre-supplementation values were within normal ranges. Mean total cholesterol in the placebo group increased from 139.9 to 153.4 mg/dL, yet decreased from 147.9 to 126.8 mg/dL and 159.2 to 131.3 mg/dL in the 200 μg chromium and 800 μg chromium groups, respectively ($p < 0.03$). Mean total cholesterol: HDL increased from 3.02 to 3.73 mg/dL in the placebo group and decreased from 3.62 to 3.37 mg/dL and 3.43 to 3.27 mg/dL in the 200 μg chromium and 800 μg chromium groups, respectively ($p < 0.04$). Since some lipid parameters were altered, while glucose tolerance was unchanged, these data support independent effects of niacin-bound chromium on glucose and lipid metabolism (Table 1) [43].

Table 1
Effect of niacin-bound chromium supplementation (NBC) on plasma, insulin, glucose, and lipid variables in male athletes

| Parameters | 200 μg NBC ($n = 12$) | | 800 μg NBC ($n = 11$) | |
|---|---|---|---|---|
| | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
| Insulin, fasted (μU/mL) | $8.9 \pm 4.2$ | $11.0 \pm 5.7$ | $6.4 \pm 2.0$ | $10.1 \pm 5.2$ |
| Insulin, post-OGTT* (μU/mL) | $54 \pm 24$ | $47 \pm 21$ | $83 \pm 66$ | $57 \pm 17$ |
| Glucose, fasted (mg/dL) | $102 \pm 8$ | $92 \pm 11$ | $98 \pm 9$ | $92 \pm 21$ |
| Glucose, post-OGTT* (mg/dL) | $112 \pm 35$ | $85 \pm 24$ | $115 \pm 31$ | $100 \pm 29$ |
| Total cholesterol (mg/dL) | $148 \pm 27$ | $127 \pm 28$ | $159 \pm 30$ | $131 \pm 33$ |
| Triglycerides (mg/dL) | $130 \pm 52$ | $102 \pm 46$ | $107 \pm 50$ | $103 \pm 33$ |

* 60 minutes after oral glucose tolerance test (OGTT); fasted = following 12-hour fast; 200 μg Cr(III) bound to 1.8 mg nicotinic acid; 800 μg Cr(III) bound to 7.2 mg nicotinic acid; data are expressed as mean ± SD.

Epidemiological studies suggest that regulation of nutritional factors, such as consumption of chromium, calcium, magnesium, and potassium, may also play a major role in regulating blood pressure [13, 41]. Nutrients and supplements that augment insulin sensitivity at the periphery, such as a soluble fiber and chromium niacin complex, also have the potential to maintain blood pressure at a significantly lower level [13]. Talpur et al. in 2003 demonstrated that treatment of aged, diabetic Zucker fatty rats with a niacin-bound chromium supplementation resulted in a lower systolic blood pressure as compared to control animals. Groups of animals received 40 μg elemental chromium as niacin-bound chromium/day, while the control group received only water for the initial 3 weeks. During weeks 4–6, the dose of niacin-bound chromium was doubled. Aged Zucker fatty rats in treatment group showed significantly lower blood pressure as compared to control [44].

Preuss et al. in 1997 examined several chromium compounds to determine its efficacy in regulating blood pressure of spontaneously hypertensive rats. The control group ingested a diet low in chromium, and five other groups were fed the same diet with various chromium compounds added at 5 ppm chloride, acetate, nicotinic acid-glycine-cysteine-glutamic acid (NA-AA), picolinate, and nicotinate. Following this, the rats were challenged with drinking water containing 5 and 10% w/v sucrose. Except for NA-AA, all chromium compounds inhibited the sucrose-induced elevation of systolic blood pressure. Acetate, picolinate, and nicotinate chromium compounds lowered HbAIC below control. Results demonstrated that high doses of chromium supplementation reduced elevated blood pressure and niacin-bound chromium ameliorated insulin resistance and prevented blood pressure elevations in spontaneously hypertensive rats [45, 46].

Niacin-bound chromium used in conjunction with other ingredients, such as zinc methionine and grape seed proanthocyanidin extract (GSPE) have also proven to effectively lower high blood pressure. In a sub-chronic study, 100 normotensive rats were

fed a diet containing supplemental niacin-bound chromium, zinc methionine, and GSPE. The rats that were on combination diets had significantly low blood pressure and lipid peroxidation in the liver and kidneys, respectively, than those fed normal diets [47]. Similarly, significant effects of niacin-bound chromium and GSPE were found on the cardiovascular parameters of normotensive and hypertensive rats. Earlier studies demonstrated the potential of niacin-bound chromium to decrease total plasma levels of cholesterol, triglycerides, and LDL cholesterol, and increase HDL. The effects of niacin-bound chromium alone and in combination with GSPE were examined in two separate investigations. Combination of niacin-bound chromium and GSPE was found to reduce vascular smooth muscle and calcium loads, thus reducing peripheral vascular resistance in insulin-resistant states [48, 49].

Prolonged use of the combination of NBC, GSPE, and zinc monomethionine has shown to markedly lower systolic blood pressure and lower HbA1C. Normotensive rats were fed a diet with NBC (5 ppm), GSPE (250 ppm), and zinc monomethionine (18 ppm elemental zinc) over a period of 1 year. Initial mean systolic blood pressures of both control and test groups were 122 mmHg. Results demonstrated that over the first 7 months, systolic blood pressure of the control animals steadily increased to 140 mmHg and remained at this level for the next 7–8 months. In contrast, the systolic blood pressure of the test animals initially decreased over the first 4 months to as low as 110–114 mmHg. The systolic blood pressure then increased over the following months, essentially reaching the starting value of 120 mmHg which was still significantly lower than control ($p < 0.001$), and HbA1C was also statistically significantly lower in the test group (5.4% vs 4.8%, $p < 0.003$). Circulating levels of cholesterol, HDL, and triglycerides were similar between the two groups [47].

High dose Cr(III) supplementation may alleviate essential hypertension due to insulin perturbations and may be a useful means to lower blood pressure [48]. Soluble fiber in the form of guar is also quite effective in favorably influencing sugar-induced systolic blood pressure elevations [46–48].

As previously discussed, ingestion of sucrose, fructose, and glucose by various rat strains is associated with perturbations in the insulin system and higher systolic blood pressure [47, 48]. However, alterations in insulin metabolism have been found in essential hypertension and many experimental forms of hypertension [48]. Preuss et al in 1995 determined that the effects of niacin-bound chromium complexes and guar, substances known to affect insulin metabolism, prevented development of sugar-induced systolic blood pressure elevations in spontaneously hypertensive rats [46].

Recent research on a combination of niacin-bound chromium and GSPE can decrease total cholesterol, LDL levels, and atherosclerosis. In a recent study, the efficacy of a combination of niacin-bound chromium and GSPE supplementation was tested in a hamster atherosclerosis model. Animals fed a hypercholesterolemic diet (HCD) of 0.2% cholesterol and 10% coconut oil. After 10 weeks of feeding HCD, the hamsters developed foam cells, which is a biomarker of early stages of atherosclerosis. Atherosclerosis (% of aorta covered with foam cells) was reduced by approximately 32%, which was observed following supplementation. Niacin-bound chromium in combination with GSPE exerted a pronounced effect on the cholesterol and triglyceride levels [50]. Preuss et al. in 2000 further demonstrated that a combination of niacin-bound chromium and GSPE can decrease total cholesterol and LDL levels significantly

Table 2
Effect of niacin-bound chromium (NBC) alone and in combination with grape seed proanthocyanidin extract (GSPE) on total cholesterol levels in hypercholesterolemic subjects

| Treatment group | Baseline (mg/dL) | Post treatment – 8 weeks (mg/dL) |
|---|---|---|
| Placebo | $251 \pm 7.6$ | $241 \pm 12.3$ |
| NBC | $267 \pm 4.4$ | $244 \pm 11.5$ |
| NBC and GSPE | $250 \pm 9.0$ | $215 \pm 11.2$ |

Data are expressed as mean ± SD.

in human volunteers. In a randomized, double-blind, placebo-controlled study, 40 hypercholesterolemic subjects with total cholesterol 210–300 mg/dL received either (Group 1) placebo bid, (Group 2) niacin-bound chromium bid (200 μg), or (Group 3) a combination of niacin-bound chromium and (Group 4) GSPE at the same dosage bid. Over 2 months, the average percent change ± SEM in the total cholesterol from baseline among groups was placebo $-3.5\% \pm 4$, chromium $-10\% \pm 5$, and combination $-16.5\% \pm 3$ ($p < 0.01$) (Table 2). The major decrease in cholesterol concentration was in the LDL levels: placebo $-3.0\% \pm 4$, chromium $-14\% \pm 4.0$, and the combination $-20\% \pm 6.0$. The combination of niacin-bound chromium and GSPE significantly decreased LDL when compared to placebo ($p < 0.01$). High-density lipoprotein levels essentially did not change among the groups. There was no significant difference in the triglyceride concentrations among the groups. Although no statistically significant differences were seen in the levels of autoantibodies to oxidized LDL, the percent changes appear to be greater in the two groups receiving GSPE as compared to the other two remaining groups. Furthermore, in five patients who started the study with elevated blood pressure (systolic BP > 140 mmHg; diastolic BP > 90 mmHg), an interesting finding occurred in one subject in the GSPE group whose initial BP decreased markedly from 166/96 mmHg to 124/83 mmHg at the end of 8 weeks (Table 3) [51].

Seventy-six patients with established atherosclerotic disease were treated daily with either 250 μg of elemental chromium orally as chromium chloride or a placebo for a period of 7–16 months. Serum chromium increased from $2.69 \pm 0.09$ to $12.12 \pm 0.77$ nmol/L (mean ± SE, $p < 0.005$). Serum triglycerides were lower ($1.68 \pm 0.11$ and $2.10 \pm 0.14$ nmol/L; $p < 0.02$) in the Cr(III)-treated patients than in the patients who received placebo, and serum HDL increased (from $0.94 \pm 0.05$ to

Table 3
Effect of niacin-bound chromium supplementation on subjects with systolic BP > 140 mmHg and diastolic BP > 90 mmHg

| Treatment group | Baseline BP (mmHg) | Post treatment – 8 weeks (mmHg) |
|---|---|---|
| Placebo | 144/75 | 122/82 |
| Niacin-bound chromium | 138/96 | 132/96 |

Value: Systolic BP/diastolic BP. Data represent the mean of three readings.

$1.14 \pm 0.07$ mmol/L, $p < 0.005$) in the patients who received Cr(III). There was no change in serum cholesterol or blood glucose during the study [52].

The effect of chromium chloride supplementation on serum lipids was tested in a double-blind, 12-week study. Twenty-three healthy adult men aged 31–60 years received either 200 μg elemental chromium (III) in 5 mL water or 5 mL plain water daily 5 days each week. At week 12 HDL increased in the chromium group from 35 to 39 mg/dL ($p < 0.05$) but did not change in the water group (34 mg/dL). Increases in HDL levels were found in those subjects having normal glucose levels together with elevated insulin levels at baseline. Overall results suggested chromium chloride raises HDL in those with evidence of insulin resistance but normal glucose tolerance [31].

During the course of a study of the hypoglycemic effects of nicotinic acid and Cr(III) on humans, two hypercholesterolemic subjects were found to experience clinically significant decreases in serum cholesterol levels. These subjects were followed for 1 year. The first subject had a cholesterol level of 10.33 mmol/L (399 mg/dL). Daily supplementation for 4 weeks with 100 mg of nicotinic acid and 200 μg of chromium chloride led to a decrease in serum cholesterol to 8.86 mmol/L (342 mg/dL). Further supplementation for 4 months led to a further decrease in serum cholesterol to 7.25 mmol/L (280 mg/dL). The second subject had a cholesterol level of 8.73 mmol/L (337 mg/dL). Four weeks of supplementation lowered the level to 6.73 mmol/L (260 mg/dL). When supplementation was discontinued, the cholesterol level rose slightly. When supplementation was reinstituted, the cholesterol level decreased to 6.68 mmol/L (258 mg/dL) [41].

The effect of chromium chloride supplementation on serum lipids and glucose tolerance was tested in another double-blind, 12-week study. Twenty-three healthy adult men aged 31–60 years received 200 μg elemental chromium daily for 5 days each week for 12 weeks. The results demonstrated that chromium supplementation raised HDL and improved insulin sensitivity [31].

Research using chromium picolinate supplementation demonstrated significant results on total cholesterol, LDL, and HDL cholesterol. In a double-blind, crossover trial, 200 μg elemental chromium as chromium picolinate was given to 14 hypercholesterolemic patients daily for 2 weeks. Total cholesterol, LDL cholesterol, and its transport protein, apolipoprotein B, were significantly decreased while levels of HDL cholesterol and its transport protein, apolipoprotein A, were beneficially raised [53].

In a diabetic rat model study, chromium picolinate has shown to significantly improve deranged carbohydrate and lipid metabolism of chemically induced diabetes in rats. The effect of 6-week treatment with chromium picolinate (8 μg/mL in drinking water) on streptozotocin-induced type 1 and type 2 diabetic rat models was studied. Treatment with chromium picolinate produced a significant decrease in elevated cholesterol and triglyceride levels in both types of diabetic rats [33]. In a similar study, male lean and obese hyperinsulinemic rats were randomly assigned to receive oral chromium picolinate 80 μg/kg body weight/day. Obese chromium picolinate-treated rats had lower plasma total cholesterol ($3.57 \pm 0.28$ vs $4.11 \pm 0.47$ mmol/L, $p < 0.05$) and higher HDL cholesterol levels ($1.92 \pm 0.09$ vs $1.37 \pm 0.36$ mmol/L, $p < 0.01$) than obese controls. Chromium picolinate did not alter plasma glucose or cholesterol levels in lean rats. Total skeletal muscle glucose transporter GLUT-4 did not differ among groups; however, chromium picolinate significantly enhanced membrane-associated GLUT-4 in obese rats after insulin stimulation. Results demonstrated that chromium picolinate supplementation

enhances insulin sensitivity and glucose disappearance, and improves lipids in male obese hyperinsulinemic (JCR:LA-corpulent) rats [54].

## TRIVALENT CHROMIUM SUPPLEMENTATION, SYNDROME X, AND WEIGHT LOSS

Obesity is a highly prevalent health problem in the United States [55, 56]. Obesity adversely affects all elements of the atherogenic cardiovascular risk profile, including blood lipids, hypertension, glucose tolerance, left ventricular hypertrophy, and plasma fibrinogen [55–57]. Existing elements of insulin resistance, obesity, hypertension, lipid disturbances, and glucose intolerance have been characterized by the term Syndrome X [55–57]. Theoretical considerations suggest that insulin resistance may be a primary factor that plays a causative role in the induction of both obesity and diabetes [55, 56]. Atherogenic risk factors associated with obesity and Syndrome X contribute independently to the development of atherosclerotic disease, and risk of a cardiovascular event also increases sharply [55, 56]. Metabolic problems such as obesity, unhealthy lipid profiles, and glucose intolerance become more common with advancing age [55]. Suboptimal chromium intake, a common prevalence in the US and Western cultures, can also contribute to these metabolic disorders [56].

Studies have shown that chromium is efficacious in maintaining proper carbohydrate and lipid metabolism in both humans and animals. Trivalent chromium's insulin signaling potential has demonstrated its effects on body composition, including reducing fat mass and increasing lean body mass. Previous studies have focused on the use of the dietary chromium, niacin-bound chromium, due to its increased absorption and retention [16].

Clinical studies have shown that chromium supplementation on overweight people induced loss of fat mass and/or increased lean body mass. Crawford et al. in 1999 demonstrated that supplementation of 600 μg of elemental chromium as niacin-bound chromium per day over a period of 2 months by African-American women with a moderate diet and exercise regimen influences weight loss and body composition [58]. In a randomized, double-blind, placebo-controlled, crossover study, 20 overweight African-American women received placebo tid during the control period and 200 μg niacin-bound chromium tid during the verum period and engaged in a modest diet–exercise regimen for 2 months. Group 1 subjects ($n = 10$) received placebo first then chromium, while Group 2 subjects ($n = 10$) received niacin-bound chromium first and placebo later. Body weights and blood chemistries were measured by routine clinical methodology. Fat and non-fat body masses were estimated using bioelectrical impedance (electrolipography) [58]. In women receiving niacin-bound chromium after the placebo period (Group 1), body weight loss was essentially the same; but fat loss was significantly greater, and non-fat body mass loss was significantly less with chromium intake. There was a significantly greater loss of fat in the placebo period compared to the verum period in the Group 2 women who received chromium first. In both groups, blood chemistries were not affected by intake of chromium for 2 months. Results confirmed that niacin-bound chromium given to African-American women in conjunction with moderate diet and exercise caused a significant loss of fat and sparing muscle compared to placebo [58].

Grant et al. in 1997 studied the effects of chromium supplementation (400 μg elemental chromium/day), as niacin-bound chromium or chromium picolinate, with or without exercise training in young, obese women [28]. Results demonstrated that exercise training combined with niacin-bound chromium supplementation resulted in significant weight loss and lowered the insulin response to an oral glucose load. Chromium picolinate supplementation resulted in significant weight gain. Furthermore, results suggested that exercise training with niacin-bound chromium supplementation is beneficial for weight loss and lower the risk of diabetes [28].

Combinations of niacin-bound chromium along with other natural ingredients such as Maitake mushroom, *Garcinia cambogia* extract, and *Gymnema sylvestre* extract have also been evaluated [44, 59, 60]. Studies have demonstrated *Garcinia cambogia*-derived (–)-hydroxycitric acid (HCA) has been shown to reduce appetite, improve serum lipid profiles, and enhance fat oxidation and decrease body weight without stimulating the central nervous system, while *Gymnema sylvestre* extract has been shown to regulate weight loss and blood sugar levels [59, 60]. In a study using a combination of niacin-bound chromium, Maitake mushroom, and HCA, results demonstrated that diabetic Zucker fatty rats had a lower maintenance of body weight compared to control animals [44]. Four groups of eight aged rats were gavaged daily with niacin-bound chromium (40 μg elemental chromium/day), Maitake mushroom (100 mg/day), and 60% (–)-hydroxycitric acid (HCA-SX, 200 mg/day) from *Garcinia cambogia*, while control group received only water. The doses of each treatment were doubled during 4–6 weeks. Animals consuming niacin-bound chromium lost approximately 9 g body weight per rat, while rats consuming Maitake mushroom lost 16 g body weight per rat. However, rats receiving HCA-SX simulated the pattern in the control group because these animals lost approximately 46 g body weight per rat [44].

In a similar study, the effect of niacin-bound chromium in combination with HCA-SX and a standardized *Gymnema sylvestre* extract on weight loss was evaluated in moderately obese subjects [59]. A randomized, double-blind, placebo-controlled human study was conducted in 60 moderately obese subjects (aged 21–50 years, BMI > 26 kg/m$^2$) for 8 weeks. One group was administered a combination of 4 mg niacin-bound chromium, 4667 mg HCA-SX, and 400 mg *Gymnema sylvestre* extract, while another group was given placebo daily in three equally divided doses 30–60 minutes before meals. All subjects received a 2000 kcal diet/day and participated in supervised walking program. At the end of 8 weeks, body weight and BMI decreased by 5–6%. Food intake, as well as total cholesterol, LDL, triglycerides, and serum leptin levels were significantly reduced, while HDL levels and excretion of urinary fat metabolites increased [59]. In another related study, 30 moderately obese subjects received the same combination of niacin-bound chromium, *Gymnema sylvestra* extract, and HCA or placebo daily in three equally divided doses 30–60 minutes before each meal for 8 weeks [60]. Subjects also received 2000 kcal diet/day and underwent a 30 minute/day supervised walking program, 5 days/week as in the previous study. Results demonstrated that at the end of 8 weeks, chromium combination supplemented group reduced body weight and BMI by 7.8 and 7.9%, respectively. Food intake was reduced by 14.1%. Total cholesterol, LDL, and triglyceride levels were reduced by 9.1, 17.9, and 18.1%, respectively, while HDL and serotonin levels increased by 20.7 and 50%, respectively. Serum leptin levels decreased by 40.5% and enhanced excretion of urinary fat metabolites increased

by 146–281%. Placebo reduced body weight and BMI by only 1.6 and 1.7%, respectively, food intake was increased by 2.8%, and LDL, triglycerides, and total cholesterol decreased by 0.8, 0.2, and 0.8%, respectively. HDL were reduced by 4.1% while serum leptin levels were increased by 0.3%, and excretion of urinary fat metabolites did not change in MDA, ACT, and FA, and marginally increased in the case of ACON [60].

In similar studies on moderately obese women participating in a 12-week exercise program, the effect of chromium picolinate supplementation on body composition, resting metabolic rate, and selected biochemical parameters in moderately obese women was investigated. In a double-blind study, 44 women (aged 27–51 years) received either 400 μg/day of elemental chromium as chromium picolinate or a placebo and participated in a supervised weight-training and walking program 2 days per week for 12 weeks. Body composition and resting metabolic rate were measured at baseline, 6 and 12 weeks. Body composition and resting metabolic rate were not significantly changed by chromium picolinate supplementation. Overall results demonstrated that 12 weeks of chromium picolinate supplementation (400 μg elemental chromium/day) did not significantly affect body composition, resting metabolic rate, plasma glucose, serum insulin, plasma glucagon, serum C-peptide, and serum lipid concentrations in moderately obese women placed on an exercise program [61].

Further research using chromium picolinate demonstrated its effects on body composition in athletes. In a double-blind study, the effects of daily chromium supplementation (200 μg elemental chromium as chromium picolinate) were investigated in football players during spring training for 9 weeks [62]. Subjects receiving chromium picolinate demonstrated urinary chromium losses five times greater than those in the placebo group. Chromium picolinate supplementation was ineffective in bringing about changes in body composition or strength during a program of intensive weight-lifting training [62]. In another double-blind, randomized, placebo-controlled study, the effects of 14 week of chromium picolinate supplementation during preseason resistance and conditioning program on body composition and neuromuscular performance in NCAA Division I wrestlers were assessed [63]. Twenty wrestlers from the University of Oklahoma was assigned to either a treatment group ($n = 7$; 20.4 years $\pm$ 0.1) receiving 200 μg elemental chromium as chromium picolinate daily, a placebo group ($n = 7$; 19.9 years $\pm$ 0.2), or a control group ($n = 6$; 20.2 years $\pm$ 0.1) using a stratified random sampling technique based on weight classification. Body composition, neuromuscular performance, metabolic performance, and serum insulin and glucose were measured before and immediately following the supplementation and training period [63]. Repeated measures ANOVA indicated no significant changes in body composition for any of the groups. Aerobic power increased significantly ($p < 0.002$) in all groups, independent of supplementation. These results demonstrated that chromium picolinate supplementation coupled with a typical preseason training program did not enhance body composition or performance variables beyond improvements seen with training alone [63].

In another double-blind, placebo-controlled study, the efficacy of chromium picolinate as a fat-reduction aid for obese individuals enrolled in a physical exercise program was investigated for 16 weeks [64]. Participants were healthy, active-duty, Navy personnel (79 men, 16 women) who exceeded the Navy's percent body fat standards of 22% fat for men, 30% for women. Mean age was 30.3 years, and comparisons between the subjects who completed the study ($n = 95$) and dropouts ($n = 109$) revealed no significant

differences in demographics or baseline percent body fat. The physical conditioning programs met a minimum of three times per week for at least 30 minutes of aerobic exercise. Subjects were given either 400 μg elemental chromium as chromium picolinate or a placebo per day [64]. At the end of 16 weeks, the group as a whole had lost a small amount of weight and body fat. However, the chromium picolinate supplemented group failed to show a significantly greater reduction in either percent body fat or body weight, or a greater increase in lean body mass than did the placebo group [64].

In a recent study, Livolsi et al. in 2001 examined the effect of chromium picolinate supplementation on muscular strength, body composition, and urinary excretion in women softball athletes [65]. Fifteen women softball athletes were randomly divided into two groups, the chromium picolinate (500 μg elemental chromium/day) treatment group ($n = 8$) and the placebo control group ($n = 7$). Results demonstrated that no significant ($p < 0.05$) differences in muscular strength or body composition were found after 6 weeks of resistance training. In addition, chromium excretion (μg per every 24 hours) was examined and increased significantly with the treatment group after the 6-week period [65].

In a longitudinal, double-blind, randomly assigned intervention study in 33 female obese subjects for 16 months with supplementation of 200 μg elemental chromium as chromium picolinate along with a very low energy diet (VLED) during the first 2 months, results show that chromium picolinate intake did not result in significant changes in blood parameters and body composition [66].

## DIETARY Cr(III) AND FREE RADICAL SCAVENGING ABILITY

Earlier studies have demonstrated perturbed glucose insulin metabolism is associated with augmented formation of free radicals [67]. Free radicals have the ability to attack vital cell components, injure cell membranes, inactivate enzymes, and damage the genetic material in the cell nucleus [67]. Augmented free-radical formation and enhanced lipid peroxidation are not uncommon in diabetes mellitus [45, 67].

Chromium(III) has been postulated to be an antioxidant, beneficial in the treatment of hypertension. Chromium(III) therapies influencing the glucose/insulin system and age-related hypertension have been shown to also lower free radical formation [45]. In spontaneously hypertensive rats, niacin-bound chromium supplementation decreased lipid peroxidation by altering free-radical formation measured, determined by thiobarbituric acid reactive substances (TBARS) [45, 46]. Preuss et al in 1997 examined several chromium compounds to determine their efficacy in regulating blood pressure of spontaneously hypertensive rats. The control group ingested a diet low in chromium, and five other groups were fed the same diet with various chromium compounds added at 5 ppm: chloride, acetate, nicotinic acid-glycine-cysteine-glutamic acid (NA-AA), picolinate, and niacin-bound chromium. Following this, the rats were challenged with drinking water containing 5% and 10% w/v sucrose. Only chromium acetate and niacin-bound chromium significantly lowered both hepatic and renal TBARS. Chromium picolinate lowered hepatic TBARS, while chromium chloride and NA-AA demonstrated no efficacy [45].

Recent studies on chromium chloride supplementation have also demonstrated antioxidant potential. Jain and Kannan in 2001 showed that chromium supplementation

prevented elevated TNF-$\alpha$ secretion and lipid peroxidation levels in $H_2O_2$-treated U937 monocytes cultured in high-glucose medium [30]. Further research on chromium chloride supplementation show estradiol + chromium chloride inhibited secretion of the pro-inflammatory cytokine interleukin (IL)-6 and oxidative stress in monocytes when exposed to high glucose. U937 human monocytes were cultured with high glucose (30 mM) with and without 17$\beta$-estradiol (0–1000 nM) and chromium chloride (0–10 $\mu$M). Results show that 17$\beta$-estradiol inhibited IL-6 and adhesion to endothelial cells ($p < 0.05$) by high glucose-treated monocytes. Treatment with 17$\beta$-estradiol + chromium chloride required a significantly lower dose of 17$\beta$-estradiol compared with 17$\beta$-estradiol alone for IL-6 inhibition. 17$\beta$-Estradiol + chromium chloride also inhibited lipid peroxidation and the adhesive potential to human endothelial cells in high glucose-treated monocytes [68]. Thus, the concept of a lower estrogen dose in combination with trivalent chromium is unique and needs further exploration in postmenopausal diabetic women.

## SAFETY OF CHROMIUM(III) COMPLEXES

Numerous studies have been conducted to evaluate the safety of trivalent chromium. In a comparative evaluation, toxicities of chromium picolinate, niacin-bound chromium and chromium chloride were examined. At equal and physiological relevant doses, chromium picolinate demonstrated mutagenic potential at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells, while at the same dose chromium chloride and niacin-bound chromium exhibited no mutagenicity [69]. In another related study, chromium picolinate was found to produce chromosome damage in Chinese hamster ovary cells 3- to 18-fold greater than control levels for soluble doses of 0.050, 0.10, 0.50, and 1.0 mM after 24-hour treatment, while no chromosome damage was observed following treatment with niacin-bound chromium or nicotinic acid [70]. The cause of damage was inferred to be due to the picolinate ligand since picolinic acid alone resulted in chromosomal damage and is clastogenic [69–71]. Although a number of published studies demonstrated the toxicity including potential neurological and other deleterious effects of chromium picolinate [72–74], however, it received self-affirmed GRAS (Generally Recognized as Safe) status recently [75].

A large body of evidence reveals enhanced bioavailability, efficacy, and safety for niacin-bound chromium [16, 28, 43–47, 49–51, 56, 73, 76]. Based on the results of extensive safety studies including acute oral, acute dermal, primary dermal, and eye irritation studies, 90-day sub-chronic toxicity, Ames' bacterial reverse mutation assay and mouse lymphoma mutagenicity assay, niacin-bound chromium received self-affirmed GRAS status [76, 77].

No significant toxic effects of chromium chloride have been reported. No detailed safety studies have been reported on other trivalent chromium complexes.

## CONCLUSION

Chromium(III), an essential trace element required for normal protein, fat, and carbohydrate metabolism, is important for energy production and plays a role in

regulating appetite, reducing sugar cravings, and increasing lean body mass [2, 4, 78]. Chromium(III) helps insulin metabolize fat, favorably influence glucose/insulin metabolism, maintains healthy cholesterol and blood sugar levels, turn protein into muscle and convert sugar into energy [2, 4, 78]. Trivalent chromium levels are known to decrease with advancing age and pregnancy, and marginal chromium deficiencies appear to be widespread [2, 78–80]. Furthermore, dietary trends that show increased consumption of more highly processed foods may lead to deficiencies of GTF and chromium in human [78].

As discussed earlier, the biologically active form of chromium, GTF, is an essential dietary agent that potentiates the action of insulin and thereby functions in regulating protein, fat, and carbohydrate metabolism, while simple trivalent chromium compounds do not [17]. Furthermore, GTF chromium extracted from Brewer's yeast is absorbed better than inorganic Cr(III) compounds [17].

In summary, based on extensive scientific research on dietary trivalent chromium, it may be concluded that supplementation of well-researched, safe trivalent chromium may be beneficial for diabetic, obese, and hypertensive population.

## REFERENCES

[1] Stohs, S. J., and Bagchi, D. (1995) Oxidative mechanisms in the toxicity of metal ions. *Free Rd.l Biol. Med. 18*, 321–336.
[2] Mertz, W. (1998) Chromium research from a distance: from 1959 to 1980. *J. Am. Coll. Nutr. 17*, 544–547.
[3] Ozawa, T., and Hanaki, A. (1990) A spin-trapping studies on the reactions of Cr(III) with hydrogen peroxide in the presence of biological reductants: Is Cr(III) nontoxic? *Biochem. Int. 22*, 343–352.
[4] Shapcott, D., and Hubert, J. (1979) *Chromium in Nutrition and Metabolism*. Elsevier North Holland Biomedical Press, Amsterdam.
[5] Lefavi, R. G., Anderson, R. A., Keith, R. E., Wilson, D., McMillan, J. L., and Stone, M. H. (1992) Efficacy of chromium supplementation in athletes: Emphasis on anabolism. *Int. J. Sports Nutr. 2*, 111–122.
[6] National Research Council. (1989) Chromium. In Recommended Dietary Allowances 10th Edition, pp. 241–284, National Academy Press, Washington, DC.
[7] Anderson, R. A., and Kozlovsky, A. S. (1985) Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr. 41*, 1177–1183.
[8] Stearns, D. M., Belbruno, J. J., and Wetterhahn, K. E. (1995) A prediction of chromium(III) accumulation in humans from chromium dietary supplements. *FASEB J. 9*, 1650–1657.
[9] Clodfelder, B. J., Emamaullee, J., Hepburn, D. D., Chakov, N. E., Nettles, H. S., and Vincent, J. B. (2001) The trail of chromium(III) in vivo from the blood to the urine: the roles of transferrin and chromodulin. *J. Biol. Inorg. Chem. 6*, 608–617.
[10] Clodfelder, B. J., Chang, C. and Vincent, J. B. (2004). Absorption of the biomimetic chromium cation triaqua-mu3-oxo-mu-hexapropionatotrichromium(III) in rats. *Biol Trace Elem Res. 98*, 159–169.
[11] Milner, J. A. (1990) Trace minerals in the nutrition of children. *J. Pediatrics 117*, S147–155.
[12] Offenbacher, E. G., and Pi-Sunyer, F. X. (1980) Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects. *Diabetes 29*, 919–925.

[13] Preuss, H. G., Jarrell, T. S., Bushehri, N., Onyejiaka, V., and Mirdamadi-Zonozi, N. (1997) Nutrients and trace elements as they effect blood pressure in the elderly. *Geriatr. Nephrol. Urol. 6*, 169–179.

[14] Feng, W., Ding, W., Qian, Q., and Chai, Z. (1998). Use of the enriched stable isotope Cr-50 as a tracer to study the metabolism of chromium (III) in normal and diabetic rats. *Biol. Trace Elem. Res. 63*, 129–138.

[15] Clodfelder, B. J., Upchurch, R. G., and Vincent, J. B. (2004). A comparison of the insulin-sensitive transport of chromium in healthy and model diabetic rats. *J. Inorg. Biochem. 98*, 522–533.

[16] Olin, K. L., Stearns, D. M., Armstrong, W. H., and Keen C. L. (1994) Comparative retention/absorption of $^{51}$chromium ($^{51}$Cr) from $^{51}$Cr chloride, $^{51}$Cr nicotinate and $^{51}$Cr picolinate in a rat model. *Trace Elem. Electrolytes 11*, 182–186.

[17] Mertz, W. (1976) Effects and metabolism of glucose tolerance factor. In *Nutrition Reviews: Present Knowledge in Nutrition*, pp. 365–372, Nutrition Foundation, Washington, DC.

[18] Cooper, J. A., Anderson, B. F., and Buckley, P. D. (1984) Structure and biological activity of nitrogen and oxygen coordinated nicotinic acid complexes of chromium. *Inorg. Chim. Acta 91*, 1–9.

[19] Glinsmann, W. H. and Mertz, W. (1966) Effect of trivalent chromium on glucose tolerance. *Metabolism 15*, 510–520.

[20] Urberg, M., and Zemel, M. B. (1987) Evidence for synergism between chromium and nicotinic acid in the control of glucose tolerance in elderly humans. *Metabolism 36*, 896–899.

[21] Li, Y. C. (1994) Effects of brewer's yeast on glucose tolerance and serum lipids in Chinese adults. *Biol. Trace Elem. Res. 41*, 341–347.

[22] Davis, C. M. and Vincent, J. B. (1997) Isolation and characterization of a biologically active chromium oligopeptide from bovine liver. *Arch. Biochem. Biophys. 339*, 335–343.

[23] Anderson, R., Cheng, N., Bryden, N. A., Polansky, M. M., Cheng, N., Chi, J., and Feng, J. (1997) Elevated intakes of supplemental chromium improves glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.

[24] Striffler, J. S., Polansky, M. M., and Anderson R. A. (1999). Overproduction of insulin in the chromium-deficient rat. *Metabolism 48*, 1063–1068.

[25] Fekete, S., Szakall, I., Kosa, E., Andrasofszky, E., Fodor, K., Hidas, A., and Tozser, J. (2001) Alteration of body composition in rats: effect of organic chromium and L-carnitine. *Acta Veterinaria Hungarica 49*, 385–398.

[26] Wilson, B. E., and Gondy, A. (1995) Effects of chromium supplementation on fasting insulin levels and lipid parameters in healthy, non-obese young subjects. *Diabetes Res. Clin. Practice 28*, 179–184.

[27] Bagchi, M., Jensen, N., Preuss, H. G., and Bagchi, D. (2004) Efficacy and toxicological assessment of a novel, niacin-bound chromium in ameliorating metabolic disorders. *10th International Congress of Toxicology-Finland 197*, 354.

[28] Grant, K. E., Chandler, R. M., Castle, A. L., and Ivy, J. L. (1997) Chromium and exercise training: effect on obese women. *Med. Sci. Sports Exerc. 29*, 992–998.

[29] Yasmin, T., Shara, M., Bagchi, M., Preuss, H. G., and Bagchi, D. (2004) Toxicological assessment of a novel niacin-bound chromium, known to ameliorate the symptoms of metabolic syndromes. *J. Am. Coll. Nutr. 492*, 77.

[30] Jain, S. K., and Kannan, K. (2001) Chromium chloride inhibits oxidative stress and TNF-alpha secretion caused by exposure to high glucose in cultured U937 monocytes. *Biochem. Biophys. Res. Commun. 289*, 687–691.

[31] Riales, R., and Albrink, M. J. (1981) Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high-density lipoprotein of adult men, *Am. J. Clin. Nutr. 34*, 2670–2678.

[32] Shinde, U. A., Sharma, G., Xu, Y. J., Dhalla, N. S., and Goyal, R. K. (2004) Anti-diabetic activity and mechanism of action of chromium chloride. *Exp. Clin. Endocrinol. Diabetes. 112*, 248–252.

[33] Shindea, U. A., Sharma, G., Xu, Y. J., Dhalla, N. S., and Goyal, R. K. (2004) Insulin sensitising action of chromium picolinate in various experimental models of diabetes mellitus, *J. Trace Elem. Med. Biol. 18*, 23–32.

[34] Cefalu, W. T., Bell-Farrow, A. D., Stegner, J., Wang, Z. Q., King, T., Morgan, T., and Terry, J. G. (1999) Effect of chromium picolinate on insulin sensitivity in vivo. *J. Trace Elem. Med. Biol. 12*, 17–83.

[35] Anderson, R. A., Cheng, N. Z., Bryden, N. A., Polansky, M. M., Cheng, N. P., Chi, J. M., and Feng, J. G. (1997) Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.

[36] Evans, G. W. (1989) The effect of chromium picolinate on insulin controlled parameters in humans, *Int. J. Biosocial Med. Res. 11*, 163–180.

[37] Rabinovitz, H., Friedensohn, A., Leibovitz, A., Gabay, G., Rocas, C., and Habot, B. (2004) Effect of chromium supplementation on blood glucose and lipid levels in type 2 diabetes mellitus elderly patients. *Int. J. VitaminNutr. Res. 74*, 178–182.

[38] Yang, X., Palanichamy, K., Ontko, A. C., Rao, M. N., Fang, C. X., Ren, J., and Sreejayan, N. (2005) A newly synthetic chromium complex—chromium(phenylalanine)3 improves insulin responsiveness and reduces whole body glucose tolerance. *FEBS Lett. 579*, 1458–1464.

[39] Abraham, A. S., Brooks, B. A., and Eylath, U. (1991) Chromium and cholesterol-induced atherosclerosis in rabbits. *Annals Nutr. Metabol. 35*, 203–207.

[40] Bolkent, S., Yanardag, R., Bolkent, S., and Doger, M. M. (2004) Beneficial effects of combined treatment with niacin and chromium on the liver of hyperlipemic rats. *Biol. Trace Elem. Res. 101*, 219–229.

[41] Urberg, M., Benyi, J., and John, R. (1988) Hypocholesterolemic effects of nicotinic acid and chromium supplementation. *J. Familiy Pract. 27*, 603–606.

[42] Zavorni, I., Bonora, E., Massimo, P., Aglio, E., Luchetti, L., Buonanno, G., Bonati, P. A., Bergonzani, M., Gnudi, L., Passeri, M., and Reaven, G. (1989) Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. *New England J. Med. 320*, 702–706.

[43] Lefavi, R. G., Wilson, D., Keith, R. E., Anderson, R. A., Blessing, D. L., Hames, C. G., and McMillan, J. L. (1993) Lipid lowering effect of a dietary chromium (III)- nicotinic acid complex in male athletes. *Nutr. Res. 13*, 239–249.

[44] Talpur, N., Echard, B. W., Yasmin, T., Bagchi, D., and Preuss, H. G. (2003) Effects of niacin-bound chromium, Maitake mushroom fraction SX and (–)-hydroxycitric acid on the metabolic syndrome in aged diabetic Zucker fatty rats *Mol. Cell. Biochem. 252*, 369–377.

[45] Preuss, H. G., Gropec, P. L., Lieberman, S., and Anderson, R. A. (1997) Effects of different compounds on blood pressure and lipid peroxidation in spontaneously hypertensive rats. *Clin. Nephrol. 47*, 325–330.

[46] Preuss, H. G., Gondall, J. A., Bustos, E., Busheri, N., Lieberman, S., Bryden, N. A., Polansky, M. M., and Anderson, R. A. (1995) Effects of chromium and guar on sugar-induced hypertension in rats. *Clin. Nephrol. 44*, 170–177.

[47] Preuss, H. G., Montamarry, S., Echard, B., Scheckenbach, R., and Bagchi, D. (2001) Long-term effects of chromium, grape seed extract, and zinc on various metabolic parameters of rats. *Mol. Cell. Biochem. 223*, 95–102.

[48] Preuss, H. G., Echard, B. W., and Manohar, V. (1997) Natural means to lessen age related hypertension and associate cardiovascular risk factors. *Internist* 14–17.

[49] Talpur, N., Echard, B., Manohar, V., Bagchi, D., and Preuss, H. (1999) Effects of chromium, grape seed extract, and zinc alone and in combination on blood pressure of spontaneously hypertensive rats. *J. Am. Coll. Nutr. 35*, 527.

[50] Vinson, J. A., Mandarano, M. A., Shuta, D. L., Bagchi, M., and Bagchi, D. (2002) Beneficial effects of a novel IH636 grape seed proanthocyanidin extract and a niacin-bound chromium in a hamster atherosclerosis model. *Mol. Cell. Biochem. 240*, 99–103.

[51] Preuss, H. G., Wallerstedt, D., Talpur, N., Tutuncuoglu, S. O., Echard, B., Myers, A., Bui, M., and Bagchi D. (2000). Effects of niacin-bound chromium and grape seed proanthocyanidin extract on the lipid profile of hypercholesterolemic subjects: a pilot study. *J. Med. 31*, 227–246.

[52] Abraham, A. S., Brooks, B. A., and Eylath, U. (1992) The effects of chromium supplementation on serum glucose and lipids in patients with and without non-insulin-dependent diabetes. *Metabolism 41*, 768–771.

[53] Press, R. I., Geller, J., and Evans, G. W. (1990) The effect of chromium picolinate on serum cholesterol and apolipoprotein fractions in human subjects. *West. J. Med. 152*, 41–45.

[54] Cefalu, W. T., Wang, Z. Q., Zhang, X. H., Baldor, L. C., and Russell, J. C. (2002) Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle Glut-4 translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. *J. Nutr. 132*, 1107–1114.

[55] Reaven, G. M. (2001) Syndrome X. *Curr. Treatment Options Cardiovascular Med. 3*, 323–332.

[56] Preuss, H. G., Bagchi, D., and Bagchi, M. (2002) Protective effects of a novel niacin-bound chromium complex and a grape seed proanthocyanidin extract on advancing age and various aspects of syndrome X. *Ann. New York Acad. Sci. 957*, 250–259.

[57] Wilson, P. W. F. and Kannel, W. B.(1998) Clustering of risk factors, obesity and syndrome X. *Nutr. Clin. Care 1*, 44–50.

[58] Crawford, V., Scheckenbach, R., and Preuss, H. G. (1999) Effects of niacin-bound chromium supplementation on body composition in overweight African-American women. *Diabetes, Obesity Metabol. 1*, 331–337.

[59] Preuss, H. G., Bagchi, D., Bagchi, M., Rao, C. V., Dey, D. K., and Satyanarayana, S. (2004) Effects of a natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and Gymnema sylvestre extract on weight loss. *Diabetes Obes Metab. 6*, 171–180.

[60] Preuss, H. G., Bagchi, D., Bagchi, M., Sanyasi Rao, C. V., Satyanarayana S., and Dey, D. K. (2004) Efficacy of a novel, natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX, niacin-bound chromium and *Gymnema sylvestre* extract in weight management in human volunteers. *Nutr. Res. 24*, 45–58.

[61] Volpe, S. L., Huang, H. W., Larpadisorn, K. and Lesser, I. I. (2001) Effect of chromium supplementation and exercise on body composition, resting metabolic rate and selected biochemical parameters in moderately obese women following an exercise program. *J. Am. Coll. Nutr. 20*, 293–306.

[62] Clancy, S. P., Clarkson, P. M., DeCheke, M. E., Nosaka, K., Freedson, P. S., Cunningham, J. J., and Valentine, B. (1994) Effects of chromium picolinate supplementation on body composition, strength, and urinary chromium loss in football players. *Int. J. Sport Nutr. 4*, 142–153.

[63] Walker, L. S., Bemben, M. G., Bemben, D. A., and Knehans, A. W. (1998) Chromium picolinate effects on body composition and muscular performance in wrestlers. *Med. Sci. Sports Exerc. 30*, 1730–1737.

[64] Trent, L. K., and Thieding-Cancel, D. (1995) Effects of chromium picolinate on body composition. *J. Sports Med. Physical Fitness 35*, 273–280.

[65] Livolsi, J. M., Adams, G. M., and Laguna, P. L. (2001) The effect of chromium picolinate on muscular strength and body composition in women athletes. *J. Strength Conditioning Res. 15*, 161–166.

[66] Pasman, W. J., Westerterp-Plantenga, M. S., and Saris, W. H. (1997) The effectiveness of long-term supplementation of carbohydrate, chromium, fibre and caffeine on weight maintenance. *Int. J. Obesity Related Metabolic Disorders 21*, 1143–1151.
[67] Warnholtz, A., Wendt, A., August, M., and Munzel, T. (2004) Clinical aspects of reactive oxygen and nitrogen species. *Biochem. Soc. Symp. 71*, 121–133.
[68] Jain, S. K., Rogier, K., Prouty, L., and Jain, S. K. (2004) Protective effects of 17beta-estradiol and trivalent chromium on interleukin-6 secretion, oxidative stress, and adhesion of monocytes: relevance to heart disease in postmenopausal women. *Free Rad. Biol. Med. 37*, 1730–1735.
[69] Stearns, D. M., Silveira, S. M., Wolf, K. K., and Luke, A. M. (2002) Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. *Mutat. Res. 513*, 135–142.
[70] Stearns, D. M., Wise, J. P., Sr., Patierno, S. R., and Wetterhahn, K. E. (1995) Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells, *FASEB J. 9*, 1643–1648.
[71] Beskid, M., Jachimowicz, J., Taraszewska, A., and Kukulska, D. (1995) Histological and ultrastructural changes in the rat brain following systemic administration of picolinic acid. *Exp. Toxicol. Pathol. 47*, 25–30.
[72] Speetjens, J. K., Collins, R. A., Vincent, J. B., and Woski, S. A. (1999) The nutritional supplement chromium(III) tris(picolinate) cleaves DNA. *Chem. Res. Toxicol. 12*, 483–487.
[73] Bagchi, D., Stohs, S. J., Downs, B. W., Bagchi, M., and Preuss, H. G. (2002). Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology 180*, 5–22.
[74] Vincent, J. B. (2003) The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent. *Sports Med. 33*, 213–230.
[75] Berner, T. O., Murphy, M. M., and Slesinski, R. (2004) Determining the safety of chromium tripicolinate for addition to foods as a nutrient supplement. *Food Chem. Toxicol. 42*, 1029–1042.
[76] Shara, M., Yasmin, T., Kincaid, A. E., Limpach, A. L., Bartz, J., Brenneman, K. A., Chatterjee, A., and Bagchi, D. (2005) Safety and toxicological evaluation of a novel niacin-bound chromium(III) complex. *J. Inorg. Biochem. 99*, 2161–2183.
[77] Personal communication from Burdock Group, Washington, DC, June 8, 2005.
[78] Preuss, H. G., and Anderson, R. A. (1998) Chromium update: examining recent literature, *Curr. Opin. Clin. Nutr. Metabol. Care 6*, 509–512.
[79] Tyson, D. A., Talpur, N. A., Echard, B. W., Bagchi, D., and Preuss, H. G. (2000) Acute effects of grape seed extract and niacin-bound chromium on cardiovascular parameters of normotensive and hypertensive rats. *Res. Commun. Mol. Pathol. Pharmacol. 5*, 91–106.
[80] Jeejeebhoy, K. N., Chu, R. C., Marliss, E. B., Greenberg, G. R., and Bruce-Robertson, A. (1977) Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am. J. Clin. Nutr. 30*, 531–538.

**Part IV**

# Toxicological effects of chromium(III)

This page intentionally left blank

*Chapter 10*

# Evaluation of chromium(III) genotoxicity with cell culture and in vitro assays

**Diane M. Stearns**

Department of Chemistry and Biochemistry, Northern Arizona University, Flagstaff, Arizona 86011-5698

The purpose of this review is to summarize the reasons supporting genotoxicity testing of $Cr^{3+}$ dietary supplements, to provide an overview of the most commonly used genotoxicity screening assays, and to review the existing data on genotoxicity studies of $Cr^{3+}$ dietary supplements, including supporting studies that shed light on possible mechanisms of action underlying the reaction of $Cr^{3+}$ with DNA.

## REASONS FOR QUESTIONING Cr(III) SUPPLEMENT SAFETY

Questions regarding the safety of Cr(III) dietary supplements are valid and necessary because these substances are not regulated by the US Food and Drug Administration (FDA), recommended doses are much higher than normal dietary intakes, the general ability of Cr(III) to damage DNA may play a role in Cr(VI)-induced cancers, and the chemistry of Cr(III) is controlled by the metal's ligand environment, thus one form of Cr(III) may be genotoxic while another produces no damage to DNA.

The general evaluation of $Cr^{3+}$ dietary supplements is important because they are taken by many people and are largely unregulated by the US government. The US FDA estimates that 158 million Americans take some form of dietary supplement. It is estimated that 10 million Americans take some form of Cr supplement [1], and in 1999 US sales of $Cr^{3+}$ supplements were reported to be second only to those of calcium [2]. Thus within the general class of dietary supplements, chromium supplements are one of the most important categories.

The Dietary Supplement Health and Education Act was signed into law in 1994, creating a category of dietary supplements separate from food and drugs, thus largely falling outside the jurisdiction of the US FDA. Currently there is no legal requirement for the US government to verify that a dietary supplement contains what is listed on the label in the stated amount or that nothing is included that is not listed on the label, nor is there a requirement that manufacturers provide evidence of safety or efficacy. However,

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

the FDA does have control over claims listed on the label. Manufacturers may not claim that a dietary supplement will diagnose, cure, mitigate, treat, or prevent a disease. They are only allowed to make general health, nutrient content, and structure/function claims.

Chromium supplements are marketed at amounts higher than normal dietary intakes for weight loss and muscle building and for regulation of blood glucose levels in non-insulin-dependent-diabetics. From 1980 to 1989, during the time Cr supplementation was gaining popularity, the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) was recommended as 50–200 μg/day [3, 4]. However, the data underlying that recommendation came from prior measurements of dietary intakes that relied on inaccurate analytical techniques. Normal chromium intakes in subjects consuming self-selected diets have been more accurately measured to be 13–56 μg Cr/day [5–7]. Those subjects showed no signs of a chromium deficiency. Recently, a new daily adequate intake (AI) has been set at 35 μg/day for males and 25 μg/day for females [8], which more accurately reflects normal dietary intakes. However, most supplements sold on the Internet or in drugstores and health stores still contain 200 μg Cr/pill with a general recommended dose of 2–3 pills per day.

Not only are recommended doses higher than dietary intakes, but much higher doses are being advocated for "maximum effects." A study of Chinese diabetics found that doses of 1000 μg Cr/day as chromium picolinate (CrPic) for 4 months decreased fasting and 2-hour glucose and insulin levels, and total plasma cholesterol [9]. A claim has been made that doses of chromium nicotinate (CrNic) at 3000–4000 μg Cr/day twice daily produce "outstanding reductions of glucose and lipid levels" in patients with type 2 diabetes [10]. A recent patent for chromium picolinate treatment of type 2 diabetics calls for 1000–10,000 mg/day [11]. Thus these supplements are being recommended at levels that are 18- to 77-fold higher than average daily dietary intakes. Diabetics, whose health is already impaired, could be ingesting relatively large amounts of metal complexes – whose actions in the body have not been completely determined – for extended periods of time. Therefore, the potential toxic effects from long-term exposures to high doses of these compounds should be investigated.

Another underlying concern regarding $Cr^{3+}$ supplements is related to the possible role of $Cr^{3+}$ in $Cr^{6+}$-induced toxicity. Details are covered elsewhere in this text, but briefly summarized here. Exposure to $Cr^{6+}$ has been shown to cause contact dermatitis [12], ulcerations of the skin and nasal mucosa [13], occupational asthma [14, 15], kidney and liver toxicity from acute exposures [16–18], and cancers of the lung [19, 20], head, neck, and nasal regions [21]. Precise molecular mechanisms underlying these endpoints are currently unknown; however, $Cr^{6+}$ is known to undergo reductive metabolism to form $Cr^{3+}$ [22], and under some conditions $Cr^{3+}$ may be oxidized intracellularly to $Cr^{6+}$ [23]. Epidemiology and case study reports come largely from occupational exposures and suicide attempts. However, the fact that industrial processing, manufacturing, and general uses of Cr compounds have created environmental contaminations [24–28] has generated interest in understanding ingestion exposures to $Cr^{6+}$ and its reduced product $Cr^{3+}$. Thus the question of $Cr^{3+}$ safety is not simply scientific, but has significant regulatory implications in terms of setting permissible exposure limits, and is therefore complicated by political and financial ramifications [29, 30].

The evaluation of toxicity of $Cr^{3+}$ supplements has revolved around questions of genotoxicity, mutagenicity, and cancer for several reasons. First, carcinogenic $Cr^{6+}$

is metabolized to $Cr^{3+}$ in the body, and $Cr^{3+}$ may be one of the ultimate species that interacts with DNA in $Cr^{6+}$-induced cancers [31, 32]. Second, the chemistry and bioavailability of $Cr^{3+}$ is altered by its coordinating ligands. For example, the $Cr^{3+}$ ion in CrPic is more bioavailable than dietary $Cr^{3+}$, $Cr^{3+}$ can accumulate in humans [33], and the coordination of $Cr^{3+}$ by aromatic ligands like picolinate can alter the chemistry of $Cr^{3+}$ making it more toxic than other forms of $Cr^{3+}$ [34].

Carcinogenicity of a chemical is ultimately defined through human epidemiology studies or animal experiments. However, these studies are relatively costly and time consuming. Therefore, several cell culture and in vitro assays have been developed as screening tools and are accepted by regulatory agencies as a first step in predicting mutagenicity or carcinogenicity of chemicals or drugs.

## JUSTIFICATION FOR USING CELL CULTURE AND IN VITRO ASSAYS

The purpose of short-term in vitro genotoxicity assays is to predict if a chemical may have a potential to cause cancer and to offer information regarding chemical mechanisms of DNA damage. These tests do not "prove" that something causes cancer, nor is there one single test that provides an unequivocal ruling that a given substance is a carcinogen. A carcinogen is generally accepted as an agent that induces neoplasms in humans or animals, increases the incidence of tumors, or speeds up the time for tumor development. Cancer is currently the second leading cause of death in the United States after heart disease. Established human carcinogens include physical agents such as UV light [35], x-radiation, and gamma radiation [36], infectious agents such as hepatitis B and C viruses [37], human papilloma viruses [38], and helicobacter pylori bacterium [39], and many chemical agents including alcohol [40], aflatoxin [41], $Cr^{6+}$ [42], and polycyclic aromatic hydrocarbons [43].

The majority of carcinogens are genotoxic (damage DNA) and mutagenic (cause a permanent, heritable change in the content or structure of DNA); therefore, most short-term assays detect DNA damage, chromosomal damage, aneuploidy (change in chromosome number), or mutations. In vitro or cell culture assays are accepted as a "first phase" of the evaluation process for predicting carcinogenicity of a chemical, and many guidelines have been issued to direct their use.

## STANDARD CELL CULTURE AND IN VITRO ASSAYS AND GUIDELINES FOR THEIR USE

The protocols and guidelines for genotoxicity and mutagenicity testing have been constantly evolving since the link between chemicals, mutagens, and carcinogens was first discovered [44–48]. The history of international regulatory agency involvement and protocol standardization has been reviewed [49]. Two of the most recent workshops devoted to international standardization of protocols were the International Workshop on Standardization of Genotoxicity Test Procedures sponsored by the International Conference on Environmental Mutagens in Melbourne in 1993 [50] and the International Workshop on Genotoxicity Testing Procedures sponsored by the Environmental Mutagen Society in Washington DC in 1999 [51]. Guidelines have also been issued through the International

Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [52], which makes recommendations for pharmaceutical testing in Europe, Japan, and the United States; the US Environmental Protection Agency (EPA) [53]; and the FDA [54]. This review will focus on the short-term in vitro assays that are commonly used to predict mutagenicity, with the understanding that in vitro testing should be combined with in vivo testing for a full evaluation of a test chemical.

The standard short-term in vitro cell culture assays are carried out in bacteria and in mammalian cells. It is generally accepted that a battery of at least three tests are required, which includes assays for bacterial mutagenesis and both mammalian chromosomal aberrations and mutagenesis [53, 55, 56]. The most common tests include the Ames assay for bacterial mutagenesis, the mammalian (hypoxanthine (guanine) phosphoribosyl transferase) *hprt* and mouse lymphoma assays for mammalian mutagenesis, and the mammalian chromosomal aberration assay. Rather than reviewing all the assays that have been used for genotoxicity screening, this review will highlight the standard tests for which published data exist for $Cr^{3+}$ supplements.

## THE AMES TEST

The Ames assay, named for developer Bruce Ames at UC Berkeley, is one of the original in vitro tests. The assay is carried out in several strains of *Salmonella typhimurium*, which have been altered by mutation so that the cells cannot synthesize histidine [57–59]. Bacteria must therefore be grown on plates that contain histidine. The assay screens for test chemicals that produce a reversion-mutation that allows bacteria to grow in the absence of histidine. Bacteria are exposed to a test compound, either by preincubation or by plate incorporation, and are plated in the presence of minimal histidine. Cells with no back-mutation will not grow beyond a faint lawn. Cells that acquire certain mutations in the previously mutated gene survive to form colonies and are referred to as "revertants". A set of bacterial strains have been mutated differently so that they are sensitive to different classes of mutagens, for example mutagens producing base substitutions or frame shifts. They have also been further modified to enhance the assay, including the presence of an *rfa* mutation that enhances permeability through the partial loss of the bacterial lipopolysaccharide wall and the ΔuvrB deletion that increases sensitivity through the loss of excision repair. The advantages of this assay are that it is inexpensive, relatively fast, and tends to test positive with known genotoxic carcinogens. For example, the Ames test has shown a 90% accuracy for testing positive with 175 known cancer-causing chemicals [47].

One shortcoming of this assay is that the Ames test is not a strong predictor of metal mutagenicity. The metals that are currently classified as human carcinogens are beryllium and beryllium compounds, cadmium and cadmium compounds, hexavalent chromium compounds, nickel compounds, lead, and the metalloid arsenic [60]. Of these metals, as well as antimony, cobalt, copper, gallium, manganese, mercury, and zinc, only hexavalent chromium has been shown to be consistently and significantly positive in the Ames test in strains TA97, TA98, TA100, TA102, TA1535, and TA1537of *S. typhimurium* [61–65]. Nickel subsulfide ($\alpha$-$Ni_3S_2$) was not active in the Ames test or the *hprt* assay in hamster V79 cells; however, it did cause chromosomal aberrations [66].

Beryllium and antimony compounds also caused sister chromatid exchange in V79 cells in the absence of bacterial mutagenicity [67].

More specifically, for the purposes of this review, $Cr^{3+}$ is generally not mutagenic in the Ames test, although some exceptions have been found when either oxidation of $Cr^{3+}$ to $Cr^{6+}$ or reduction of $Cr^{3+}$ to $Cr^{2+}$ occurs. For example, the $Cr^{3+}$ compounds chromium potassium sulfate, chromium nitrate, chromium chloride, neochromium, and chromium alum became mutagenic in *S. typhimurium* strain TA100 when co-treated with potassium permanganate [68]. Also, compounds of $Cr^{3+}$ with bipyridine and phenanthroline were found to be mutagenic in strains TA102 and TA2638, and a Fenton-type mechanism, with oxygen radicals proposed to being the ultimate mutagenic species, was determined through cyclic voltametry [69, 70]. Results of Ames testing of specific $Cr^{3+}$ compounds and supplements are presented later in this chapter.

## THE CHROMOSOMAL ABERRATION ASSAY

Chromosomal aberrations, or visible changes to chromosome structure and morphology, are also used to predict carcinogenicity [71–73]. The chromosomal aberration assay measures the ability of a test chemical to be clastogenic, which is a term that is derived from the Greek word "clastos" meaning "broken". Common mammalian cells include Chinese hamster ovary (CHO) cells, Chinese hamster V79 lung cells, and human or rat lymphocytes. Cells are exposed to a test chemical for a given time, then treated briefly with colcimid to arrest cells in metaphase. Cells are isolated, swelled in a hypotonic buffer, and fixed, stained, and analyzed under a microscope. Aberrations are generally classified into four main categories: chromosome breaks, chromosome exchanges, chromatid breaks, and chromatid exchanges; although more extensive classifications exist [74, 75].

## THE *hprt* ASSAY

The hypoxanthine (guanine) phosphoribosyl transferase (*hprt*) assay measures the ability of a test chemical to cause mutations at the *hprt* locus in mammalian cells. The *hprt* gene codes for the HPRT enzyme. This gene is located on the X chromosome and is hemizygous in CHO cells ($hprt^{+/-}$). This enzyme is involved in the purine recycling pathway. It catalyzes the reaction of hypoxanthine or guanine with 5-phospho-a-D-ribosyl-1-pyrophosphate (PRPP) to produce either inosine 5′-monophosphate or guanosine 5′-monophosphate. Its use in mutagen screening derives from the fact that the poison 6-thioguanine (6-TG) is also a substrate for the HPRT enzyme. The phosphorylation of 6-TG by a functional HPRT enzyme leads to incorporation of 6-TG into DNA, which kills the cell. Mutant cells that have lost a functional HPRT enzyme will survive in the presence of 6-TG, and non-mutated cells will die. Thus the *hprt* assay selects for any mutation that produces a non-functional enzyme, including base substitutions and major deletions.

## THE IN VITRO MOUSE LYMPHOMA ASSAY

The mouse lymphoma assay is a mammalian mutation test that measures forward mutations in the thymidine kinase locus of L5178Y mouse lymphoma cells. Thymidine kinase (*tk*) uses ATP to phosphorylate thymidine producing thymidine 5′-phosphate, which can then be incorporated into DNA. Normal cells contain two copies of the *tk* gene ($tk^{+/+}$), but the cell line used in this assay has a mutation that has made one allele nonfunctional ($tk^{+/-}$). In a manner similar to the *hprt* assay, a cell with a functional *tk* gene, and therefore functional tk enzyme, will incorporate the thymidine analog trifluorothymidine (TFT) into its DNA and will die. A cell that generates a mutation in the remaining *tk* allele that makes the protein nonfunctional ($tk^{-/-}$) will survive in the presence of TFT. Thus similar to 6-TG in the *hprt* assay, TFT will select for cells with *tk* mutations.

## STRENGTHS AND WEAKNESSES OF IN VITRO ASSAYS

The strengths of in vitro assays lie in the similarities between bacterial, animal, and human DNA, and the ability of DNA to react with directly genotoxic chemicals. The assays are less expensive and faster to perform than animal experiments, and initial screenings with in vitro tests contribute to scientists' ability to decrease unnecessary animal testing.

Weaknesses in these assays include the existence of both false positives and false negatives. False positives can occur when there are differences in absorption, distribution, metabolism, excretion, and DNA repair capabilities among humans, animals, or cells grown in culture. Addition of S9 subfraction from rat or human liver microsomes is commonly used for metabolic activation or deactivation of chemicals in in vitro assays; however, this provides only a first approximation of in vivo metabolism.

Also, it is necessary to use a battery of tests because no single test can detect all genotoxins or mutagens. For example, analysis of 951 chemicals found that 23% of established carcinogens tested negative in a standard battery of tests in mammalian cells [76]. Similarly, the mouse lymphoma assay produced negative results with 22% of known carcinogens [77].

Lastly, a chemical does not have to be directly DNA damaging to cause cancer. There are chemicals that are not genotoxic or mutagenic but are still carcinogenic. Examples include hormones [78], immunosuppressants [79], the metalloid arsenic [80], and cadmium [81] with mechanisms beyond the scope of this review. Epigenetic carcinogens that do not cause direct DNA damage are not detected by genotoxicity and mutagenicity assays. The $Cr^{3+}$ compounds commonly used in dietary supplements have not been analyzed for epigenetic mechanisms, with the exception of chromic chloride (vide infra); however, they have been screened to some extent in short-term in vitro assays for mutagenicity and clastogenicity, with varying results. The most commonly studied supplements have been chromium nicotinate, chromic chloride, and chromium picolinate.

## RESULTS FOR CHROMIUM(III) NICOTINATE

The compound chromium nicotinate (CrNic), resulting from coordination of $Cr^{3+}$ with nicotinic acid, is not well characterized. No X-ray structural determination has been

carried out. Reaction of two or three equivalents of nicotinic acid with $Cr^{3+}$ produces a non-crystalline solid above pH 7.5 with a 2 : 1 nicotinate to $Cr^{3+}$ ratio [82], and it is presumed to exist as a polymer with $Cr^{3+}$ atoms bridged through hydroxyl groups [83].

Chromium nicotinate has demonstrated a low toxicity. For example, to date only one adverse clinical case report exists, describing chromium polynicotinate-induced hepatitis [84]. It is also less active in cell culture assays. Chromium nicotinate did not produce cell death at concentrations up to 1 mM $Cr^{3+}$, nor did it produce chromosomal aberrations in CHO cells after 24 hours of exposure [85]. One study reported that CrNic produced less oxidative stress and DNA damage than CrPic [86]; however, that work included co-authors from the company that manufactures CrNic (Interhealth), and data presented in the text of the report showed that both CrPic and CrNic actually produced equivalent levels of cytochrome c reduction, formation of 2,3- and 2,5-dihydroxybenzoic acids, and DNA fragmentation in J774A.1 macrophages that were above untreated controls after 24 hours of exposure. At this time there are no published reports on mutagenicity testing of CrNic.

## RESULTS FOR CHROMIC CHLORIDE

Chromic chloride hexahydrate ($CrCl_3 \cdot 6H_2O$) exists as the trans-isomer, with the $Cr^{3+}$ atom bound by two chloride atoms and four water molecules. It has historically been used as a standard $Cr^{3+}$ source to compare $Cr^{3+}$ activity to that of $Cr^{6+}$. It is generally, but not exclusively, negative in cell culture genotoxicity tests.

Chromic chloride did not cause DNA damage in V79 hamster cells that could be detected by alkaline elution, nor did it stimulate DNA repair in human EUE cells or in diploid human fibroblasts. It was negative in the Ames test in TA98, TA100, TA1535, and TA1538 strains, it did not produce sister chromatid exchange in CHO cells, nor was it able to transform BHK Syrian hamster fibroblasts to grow in soft agar [87]. Chromic chloride did not cause cytotoxicity, chromosomal aberrations, or *hprt* mutations in CHO cells [85, 88].

In contrast to in vitro assays, chromic chloride has been shown to have epigenetic mechanisms leading to preconceptional and transgenerational carcinogenesis, which may be related to its ability to influence gene expression and endocrine disruption. Male NIH Swiss mice given preconception exposure to chromic chloride produced offspring with pheochromocytomas, lung tumors, thyroid follicular cell and Harderian gland tumors, ovarian cysts, uterine abnormalities, male reproductive gland tumors, and renal non-neoplastic lesions [89]. Growing evidence suggests chromic chloride exposure may affect gene expression and endocrine disruption [90–92].

## RESULTS FOR OTHER Cr(III) SUPPLEMENTS

Chromium(III) has been proposed to exist in a trinuclear core in the low-molecular-weight chromium-binding substance, which has been postulated to be the biologically active form of essential $Cr^{3+}$ in vivo [93]. The propionate oxotrimer cation, $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ has been offered as both a model and a biologically

relevant form of Cr(III) for nutritional supplementation and therapeutics [94]. It has not yet been extensively tested for in vitro genotoxicity.

One study has been reported in which the nitrate salt of the propionate oxotrimer did not cause DNA strand breaks in pUC19 plasmid DNA either by itself or in the presence of ascorbate; however, in the presence of 210 μM $H_2O_2$ a solution of 120 μM oxotrimer did cause plasmid relaxation [95]. This observation was consistent with a study from the same laboratory that found plasmid DNA strand breaks with solutions of the acetate oxotrimer and $H_2O_2$ [96].

## RESULTS FOR Cr(III) PICOLINATE

The dietary supplement chromium trispicolinate (CrPic) has been the most commercially successful chromium supplement. Chromium picolinate exists as a neutral 3 : 1, picolinate : $Cr^{3+}$ complex. It was first patented for use as a dietary supplement in 1982 [97]. It can be synthesized by aqueous reaction of chromic chloride with three equivalents of picolinic acid [98]. At $Cr^{3+}$ concentrations of 0.30 M, filtered solutions left at 37 °C crystallize over ~24 hours to yield $Cr(pic)_3$-$H_2O$ at ~98% purity and ~50% yield. It is also commercially available. It was structurally characterized by x-ray crystallography in 1992 [99]. CrPic has been shown to be clastogenic, mutagenic, and genotoxic in some, but not all, of the standard assays that are used to predict carcinogens.

The first study to raise the question of the potential toxicity of CrPic demonstrated that it produced chromosomal aberrations in CHO AA8 cells, while equivalent doses of chromium nicotinate, chromic chloride, or picolinate in the absence of $Cr^{3+}$ were not active [85]. The dose range was chosen based on the exposure range that showed cytotoxicity by colony formation, with the highest dose tested producing 69% cell survival at 24-hour exposures. The compound was tested in particulate form, as a suspension in acetone, at doses of 4–40 μg/cm$^2$, which corresponded to 0.050–0.50 mM CrPic. The compound was originally tested as an acetone suspension because it is weakly soluble in water and organic solvents, except for DMSO in which 50 mM solutions can be obtained; however, the radical scavenger DMSO is not generally recommended for the chromosomal aberration assay [100] (vide infra). Clastogenicity was also measured for CrPic in soluble form, which may have contained 1 : 1 and 2 : 1 picolinate:$Cr^{3+}$ complexes, at a range of 0.025–1.0 mM CrPic. However, the level of chromosomal damage was the same for equivalent doses of soluble and insoluble CrPic. The lowest doses of CrPic that were active were 8 μg/cm$^2$ for insoluble CrPic and 0.050 mM for soluble CrPic. Equivalent doses of free picolinate were not clastogenic, but chromosomal aberrations were observed with higher doses of 1.5 and 2.0 mM free picolinate.

A subsequent study funded by the manufacturer of CrPic did not find chromosomal aberrations induced by solutions of CrPic in DMSO at 4 or 20 hours of exposure [101]. The relevance of DMSO as a solvent for CrPic is discussed below.

An in vitro study has shed light on a potential mechanism for CrPic-induced DNA damage. Chromium picolinate was shown to cause DNA single-strand breaks in pUC19 plasmid DNA in the presence of ascorbate (vitamin C), dithiothreitol, or hydrogen peroxide [102]. It was inferred that coordination of $Cr^{3+}$ by picolinate shifted the III/II redox potential such that the $Cr^{3+}$ could be reduced by ascorbate or thiols

and air oxidized to catalytically generate reactive oxygen species through a Fenton-type mechanism.

Generation of DNA and chromosomal damage is one of the first steps toward carcinogenicity. However, if a chemical causes DNA damage, but either the DNA is repaired or the cell undergoes apoptosis, then there is no risk for cell transformation. However, unrepaired DNA damage that is translated to a mutation produces a change in the genetic code that is passed on through cell division, and mutations may lead to changes in cell growth regulation that transform cells. Therefore, mutagenicity assays are an important component of genotoxicity screening. Three independent studies have shown that CrPic is mutagenic.

Chromium picolinate was first found to be mutagenic at the *hprt* locus in CHO cells [88]. It was tested as the insoluble form in an acetone suspension at doses from 20 to 80 $\mu g/cm^2$ for 48 hours. The 80 $\mu g/cm^2$ dose produced a mutation frequency of 58 per $10^6$ surviving cells, or an average 40-fold increase in *hprt* mutants relative to untreated cells. Equivalent doses of free picolinate were more cytotoxic than CrPic, yet produced no *hprt* mutants. Equivalent doses of chromic chloride were not cytotoxic and produced no substantial *hprt* mutants. Data were interpreted to suggest that coordination of $Cr^{3+}$ with picolinate produced a complex that was more genotoxic than $Cr^{3+}$ or picolinate alone.

A recent study, funded by the manufacturers of CrPic, found that CrPic was not mutagenic in the same assay if a shorter 5-hour exposure time was used, or if the complex was dissolved in DMSO [103]. These results are consistent with the well-established observations that (1) uptake of $Cr^{3+}$ complexes are relatively slow due to the fact that there is no active transport pathway available [22], thus shorter exposure times will result in less of the genotoxic substance entering the cell, and (2) co-treatment of CrPic with DMSO, a solvent well-known to be a radical scavenger [104], should decrease the reactive oxygen species produced through Fenton-type chemistry [34], thus decreasing the DNA damage that leads to mutations. Thus the manufacturer's study did not negate the original report.

In contrast to the manufacturer's study, the ability of CrPic to be mutagenic has been independently confirmed in two separate studies. Mutagenicity of CrPic was observed in *Drosophila melanogaster* by the Vincent laboratory [105]. Exposure of *Drosophila* to CrPic at levels of up to 260 $\mu g$ Cr/kg medium decreased the number of progeny reaching the pupal stage of development, reduced successful eclosion rates, and produced developmental delays and germ-line mutations. Chromic chloride was inactive, but picolinate showed similar effects as CrPic in terms of appearance in total pupal cases, eclosion of adult progeny, and percentage of unhatched pupae.

A recent study by the FDA and the National Cancer Institute found CrPic to be negative in the Ames test, but positive in the mouse lymphoma assay [106]. Exposure of *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 produced no significant reversion mutants with solutions of CrPic in DMSO, or aqueous solutions of Pic or chromic chloride. However, exposure of L5178Y mouse lymphoma cells to aqueous solutions of CrPic up to 1000 $\mu g$/mL (124 $\mu g$/mL $Cr^{3+}$ or 2.4 mM CrPic) for 4 hours resulted in trifluorothymidine-resistant colonies at mutation frequencies (MF, or difference from background) of 315 and 327 colonies/per $10^6$ survivors without and with S9 activation, respectively. A positive response was defined as an MF of at least 100

mutant colonies per $10^6$ surviving cells above background. The second highest dose of 500 μg/mL (62 μg/mL $Cr^{3+}$ or 1.2 mM CrPic) was also positive, with an MF of 295 and 177 colonies/per $10^6$ survivors without and with S9 activation, respectively.

The observations that CrPic is clastogenic [85], mutagenic [88, 105, 106], and causes DNA strand breaks in isolated DNA [102] predict that CrPic should be directly DNA damaging in cultured cells. This hypothesis has been supported through the characterization of 61 CrPic-induced 6-thioguanine-resistant mutants. DNA sequencing showed that base substitutions comprised 33% of the mutations, with transversions being predominant; deletions made up 62% of the mutations, with one exon deletions predominant; and 1–4 base pair insertions made up 5% of the characterized mutations [107]. Chromium picolinate induced a statistically greater number of deletions and a statistically smaller number of base substitutions that have been measured in spontaneously generated mutants [108]. Further support for direct DNA damage was provided by comet assay analysis of DNA lesions induced by CrPic in CHO cells, which showed evidence of DNA strand breaks, DNA crosslinks, and oxidative damage (Lencinas et al., in preparation).

As a result of the preliminary cell culture and in vitro experiments, CrPic was nominated in 1998 for evaluation and long-term carcinogenicity testing by the National Toxicology Program (NTP) and was given high priority. Rodent studies are in progress.

Current data from the NTP suggest that understanding the metabolism of CrPic will also be necessary to fully evaluate its biological activity. A report on the disposition and metabolism of [$^{14}$C]-CrPic in rats and mice has been released [109]. The $^{14}$C radiolabel was randomly distributed across the pyridine ring. Chromium picolinate was found to be stable to rat stomach and small intestine contents in vitro, which was consistent with a previous study [110]. It was inferred that the complex disassociated at or near the intestinal wall, and the absorbed picolinic acid was excreted mostly as *N*-picolylglycine; however, ~8–10% of the radiolabel was excreted from the breath as $CO_2$, plus an unidentified major metabolite was observed in mice along with *N*-picolylglycine. It is not currently known if any of the picolinate metabolism products induce toxicity. Absorption of $Cr^{3+}$ was on the order of a few percent in both rats and mice after a single oral dose, consistent with previous estimates [33]. Adipose, blood, and liver showed Cr levels 2–6 times higher than untreated animals, with liver containing the highest amounts. The observation that approximately 30% of Cr detected in rat liver was measured as the intact CrPic complex justifies further investigation of CrPic mutagenicity and the mechanisms underlying CrPic-induced DNA damage.

## SUMMARY AND CONCLUSIONS

In summary, of all the $Cr^{3+}$ dietary supplements that have been tested for genotoxicity and mutagenicity to date, CrPic is the most active. The dietary supplement CrPic is genotoxic, clastogenic, and mutagenic in short-term assays, and thus far the only studies attempting to refute the published reports on genotoxicity were funded by the supplement manufacturer. In vivo testing, including the results of the NTP's long-term carcinogenicity studies in rodents, will offer further information for risk assessment; however, further elucidation of the molecular mechanisms behind the biochemistry of CrPic will also be needed to evaluate the data as a whole.

Lastly, negative results in standard in vitro assays do not guarantee the safety of $Cr^{3+}$ supplements. For example, chromic chloride is often negative in vitro, yet in animals it is a preconceptional and transgenerational carcinogen that acts through epigenetic mechanisms. Other $Cr^{3+}$ compounds have not been tested for epigenetic activity. In light of growing evidence of epigenetic $Cr^{3+}$ toxicity, in vivo testing of other forms of $Cr^{3+}$ dietary supplements is also necessary before supraphysiological doses of $Cr^{3+}$ compounds should be recommended for weight loss or diabetes treatment.

## REFERENCES

[1] Mirasol, F. (2000) Chromium picolinate market sees robust growth and high demand. *Chem. Market Rep. 257*, 26.

[2] Nielsen, F. H. (1996) Controversial chromium: does the superstar mineral of the mountebanks receive appropriate attention from clinicians and nutritionists? *Nutr. Today 31*, 226–233.

[3] National Research Council (1980) *Recommended Dietary Allowances, 9th Ed. Report of the Committee on Dietary Allowances, Division of Biological Sciences, Assembly of Life Science, Food and Nutrition Board, Commission on Life Science, National Research Council.* National Academy Press, Washington, D.C.

[4] National Research Council (1989) *Recommended Dietary Allowances, 10th Ed. Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Commission on Life Science, National Research Council*. National Academy Press, Washington, D.C.

[5] Bunker, V. W., Lawson, M. S., Delvees, H. T., and Clayton, B. E. (1984) The uptake and excretion of chromium by the elderly. *Am. J. Clin. Nutr. 39*, 797–802.

[6] Anderson, R. A., and Kozlovsky, A. S. (1985) Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am. J. Clin. Nutr. 41*, 1177–1183.

[7] Offenbacher, E. G., Spencer, H., Dowling, H. J., and Pi-Sunyer, F. X. (1986) Metabolic chromium balances in men. *Am. J. Clin. Nutr. 44*, 77–82.

[8] National Research Council (2002) *Dietary Reference Intakes for Vitamin A, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. A Report of the Panel on Micronutrients, Subcommittee on Upper Reference Levels of Nutrients and of Interpretations and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes.* National Academy of Sciences, Washington, D.C.

[9] Anderson, R. A., Cheng, N., Bryden, N. A., Polansky, M. M., Cheng, N., Chi, J., and Feng, J. (1997) Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.

[10] Lamson, D. W., and Plaza, S. M. (2002) The safety and efficacy of high-dose chromium. *Alt. Med. Rev. 7*, 218–235.

[11] McCarty, M. F. (2001) US Patent No. 6,329,361. High-dose chromic picolinate treatment of type II diabetes.

[12] Hansen, M. B., Rydin, S., Menne, T., and Duus, J. J. (2002) Quantitative aspects of contact allergy to chromium and exposure to chrome-tanned leather. *Contact Dermatitis 47*, 127–134.

[13] Gibb, H. J., Lees, P. S., Pinsky, P. F., and Rooney, B. C. (2000) Clinical findings of irritation among chromium chemical production workers. *Am. J. Ind. Med. 38*, 127–131.

[14] Park, H. S., Yu, H. J., and Jung, K. S. (1994) Occupational asthma caused by chromium. *Clin. Exp. Allergy 24*, 676–681.

[15] Bright, P., Burge, P. S., O'Hickey, S. P., Gannon, P. F., Robertson, A. S., and Boran, A. (1997) Occupational asthma due to chrome and nickel electroplating. *Thorax 52*, 28–32.
[16] Wedeen, R. P., and Qian, L. F. (1991) Chromium-induced kidney disease. *Environ. Health Perspect. 92*, 71–74.
[17] Loubieres, Y., de Lassence, A., Bernier, M., Viellard-Baron, A., Schmitt, J. M., Page B., and Jardin, F. (1999) Acute, fatal, oral chromic acid poisoning. *J. Toxicol. Clin. Toxicol. 37*, 333–336.
[18] Stift, A., Fridl, J., Langle, F., Berlakovich, G., Steininger, R., and Muhlbacher, F. (2000) Successful treatment of a patient suffering from severe acute potassium dichromate poisoning with liver transplantation. *Transplantation 69*, 2454–2455.
[19] International Agency for Research on Cancer, IARC (1990) Chromium and chromium compounds. *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Chromium, Nickel and Welding*. International Agency for Research on Cancer, Lyon France, pp. 49–256.
[20] National Toxicology Program (2005) *Chromium Hexavalent Compounds. 11th Report on Carcinogens*. Department of Health and Human Services, Washington, D.C.
[21] Satoh, N., Fukuda, S., Takizawa, M., Furuta, Y., Kashiwamura M., and Inuyama, Y. (1994) Chromium-induced carcinoma in the nasal region. A report of four cases. *Rhinology 32*, 47–50.
[22] Jennette, K. W. (1979) Chromate metabolism in liver microsomes. *Biol. Trace Elem. Res. 1*, 55–62.
[23] Mulyani, I., Levina, A., and Lay, P. A. (2004) Biomimetic oxidation of chromium(III): Does the antidiabetic activity of chromium(III) involve carcinogenic chromium(VI)? *Andew. Chem. Int. Ed. Engl. 27*, 4504–4507.
[24] LaValle, J. M. (1991) Mechanisms of toxicity/carcinogenicity and Superfund decisions. *Environ. Health Perspect. 92*, 127–130.
[25] Durant, J. L., Chen, J., Hemond, H. F., and Thilly, W. G. (1995) Elevated incidence of childhood leukemia in Woburn, Massachusetts: IEHS Superfund Basic Research Program searches for causes. *Environ. Health Perspect. 103 (Suppl. 6)*, 93–98.
[26] Fay, R. M., and Muntaz, M. M. (1996) Development of a priority list of chemical mixtures occurring at 1188 hazardous waste sites, using the HazDat database. *Food Chem. Toxicol. 34*, 1163–1165.
[27] Fagliano, J. A., Savrin, J., Udasin, I., and Gochfeld, M. (1997) Community exposure and medical screening near chromium waste sites in New Jersey. *Regul. Toxicol. Pharmacol. 26*, S13–S22.
[28] Renner, R. (2004) Huge chromium plume threatens Colorado River. *Environ. Sci. Technol. 38*, 178A.
[29] Renner, R. (2003) Allegations of manipulation lead to withdrawal of Cr(VI) report. *Environ. Sci. Technol. 37*, 276A–277A.
[30] Steinpress, M. G., and Ward, A. C. (2001) The scientific process and Hollywood: the case of hexavalent chromium. *Ground Water 39*, 321–322.
[31] O'Brien, T. J., Ceryak, S., and Patierno, S. R. (2003) Complexities of chromium carcinogenesis: Role of cellular response, repair and recovery mechanisms. *Mutat. Res. 533*, 3–36.
[32] Zhitkovich, A. (2005) Importance of chromium-DNA adducts in mutagenicity and toxicity of chromium(VI). *Chem. Res. Toxicol. 18*, 3–11.
[33] Stearns, D. M., BelBruno, J. J., and Wetterhahn, K. E. (1995) A prediction of Cr(III) accumulation in humans from ingestion of Cr dietary supplements. *FASEB J. 9*, 1650–1657.
[34] Speetjens, J. K., Collins, R. A., Vincent, J. B., and Woski, S. A. (1999) The nutritional supplement chromium(III) tris(picolinate) cleaves DNA. *Chem. Res. Toxicol. 12*, 483–487.

[35] Ramirez, C. C., Federman, D. G., and Kirsner, R. S. (2005) Skin cancer as an occupational disease: The effect of ultraviolet and other forms of radiation. *Int. J. Dermatol. 44*, 95–100.
[36] (No Authors) (2000) IARC working group on the evaluation of carcinogenic risks to humans: Ionizing radiation, Part I, X-gamma-radiation and neutrons. Lyn, France, 26 May–2 June 1999. *IARC Monogr. Eval. Carcinogen. Risks Hum. 75 (Part I)* 1–448.
[37] Bosch, F. X., Ribes, J., Cleries, R., and Diaz, M. (2005) Epidemiology of hepatocellular carcinoma. *Clin. Liver Dis. 9*, 191–211.
[38] Gatza, M. L., Chandhasin, C., Ducu, R. I., and Marriott, S. J. (2005) Impact of transforming viruses on cellular mutagenesis, genome stability, and cellular transformation. *Environ. Mol. Mutagen. 45*, 304–325.
[39] Leodolter, A., Naumann, M., and Malfertheiner, P. (2004) Prevention of gastric cancer by Helicobacter pylori eradication. *Dig. Dis. 22*, 313–319.
[40] Voigt, M. D. (2005) Alcohol in hepatocellular cancer. *Clin. Liver. Dis. 9*, 151–169.
[41] Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., and Aggarwal, D. (2004) Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin. Nutr. 80*, 1106–1122.
[42] Costa, M. (2003) Potential hazards of hexavalent chromate in our drinking water. *Toxicol. Appl. Pharamacol. 188*, 1–5.
[43] Xue, W., and Warshawsky, D. (2005) Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: A review. *Toxicol. Appl. Pharmacol. 206*, 73–93.
[44] Auerbach, J. M., and Robson J. M. (1946) Chemical production of mutations. *Nature 157*, 302.
[45] Lawley, P. D. (1966) Effects of some chemical mutagens and carcinogens on nucleic acids. *Prog. Nucleic Acid Res. Mol. Biol. 5*, 89–131.
[46] Auerbach, C. (1967) The chemical production of mutations. The effect of chemical mutagens on cells and their genetic material is discussed. *Science 158*, 1141–1147.
[47] McCann, J., and Ames, B. N. (1976) Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals: Discussion. *Proc. Natl. Acad. Sci. USA 73*, 950–954.
[48] Weisburger, J. H. (1999) Carcinogenicity and mutagenicity testing, then and now. *Mutat. Res. 437*, 105–112.
[49] MacGregor J. T., Casciano, D., and Muller, L. (2000) Strategies and testing methods for identifying mutagenic risks. *Mutat. Res. 455*, 3–20.
[50] Kirkland, D. J., ed. The "International Workshop on Standardization of Genotoxicity Test Procedures." In *Mutation Research, 312* 3, 1994, pp. 195–321.
[51] Kirkland, D. J., ed. The International Workshop on Genotoxicity Test Procedures." In *Environmental and Molecular Mutagenesis* volume 35 issue 3, 2000, pp. 159–263.
[52] Muller, L., Kikuchi, Y., Probst, G., Schechtman, L., Shimada, H., Sofuni, T., and Tweats, D. (1999) ICH-harmonised guidances on genotoxicity testing of pharmaceuticals: evolution, reasoning and impact. *Mutat. Res. 436*, 195–225.
[53] Dearfield, K. L., Cimino, M. C., McCarroll, N. E., Mauer, I., and Valcovic, L. R. (2002) Genotoxicity risk assessment: A proposed classification strategy. *Mutat. Res. 521*, 121–135.
[54] FDA/CFSAN (2004) Redbook 2000 Toxicological principles for the Safety Assessment of Food Ingredients.
[55] Brambilla, G., and Martelli, A. (2004) Failure of the standard battery of short-term tests in detecting some rodent and human carcinogens. *Toxicology 196*, 1–19.
[56] Snyder, R. D., and Green, J. W. (2001) A review of the genotoxicity of marketed pharmaceuticals. *Mutat. Res. 488*, 151–169.
[57] Maron, D. M., and Ames, B. N. (1983) Revised methods for the Salmonella mutagenicity test. *Mutat. Res. 113*, 173–215.

[58] Gee, P., Maron, D. M., and Ames, B. N. (1994) Detection and classification of mutagens: a set of base-specific Salmonella tester strains. *Proc. Natl. Acad. Sci. USA 91*, 11606–11610.
[59] Mortelmans, K., and Zeiger, E. (2000) The Ames Salmonella/microsome mutagenicity assay. *Mutat. Res. 455*, 29–60.
[60] National Toxicology Program (2005) *11th Report on Carcinogens*. Department of Health and Human Services, Washington, D.C.
[61] Petrilli, F. L., and De Flora, S. (1977) Toxicity and mutagenicity of hexavalent chromium on Salmonella typhimurium. *Appl. Environ. Microbiol. 33*, 805–809.
[62] Tso, W. W., and Fung, W. P. (1981) Mutagenicity of metallic cations. *Toxicol. Lett. 8*, 195–200.
[63] De Flora, S., Camoirano, A., Zanacchi, P., and Bennicelli, C. (1984) Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds, negative with other Salmonella strains. *Mutat. Res. 134*, 159–165.
[64] Leonard, A. (1988) Mechanisms in metal genotoxicity: The significance of in vitro approaches. *Mutat. Res. 198*, 321–326.
[65] Codina, J. C., Perez-Torrente, C., Perez-Garcia, A., Cazorla, F. M., and de Vicente, A. (1995) Comparison of microbial tests for the detection of heavy metal genotoxicity. *Arch. Environ. Contam. Toxicol. 29*, 260–265.
[66] Arrouijal, F. Z., Hildebrand, H. F., Vophi, H., and Marzin, D. (1990) Genotoxic activity of nickel subsulphide alpha-$Ni_3S_2$. *Mutagenesis 5*, 583–589.
[67] Kuroda, K., Endo, G., Okamoto, A., Yoo, Y. S., and Horiguchi, S. (1991) Genotoxicity of beryllium, gallium and antimony in short-term assays. *Mutat. Res. 264*, 163–170.
[68] Petrilli, F. L., and de Flora, S. (1978) Oxidation of inactive trivalent chromium to the mutagenic hexavalent form. *Mutat. Res. 58*, 167–173.
[69] Sugden, K. D., Burris, R. B., and Rogers, S. J. (1990) An oxygen dependence in chromium mutagenesis. *Mutat. Res. 244*, 239–244.
[70] Sugden, K. D., Geer, R. D., and Rogers, S. J. (1992) Oxygen radical-mediated DNA damage by redox-active Cr(III) complexes. *Biochemistry 31*, 11626–11631.
[71] Knudson, A. G. (2001) Two genetic hits (more or less) to cancer. *Nat. Rev. Cancer 1*, 157–162.
[72] Bonassi, S., Znaor, A., Norppa, H., and Hagmar, L. (2004) Chromosomal aberrations and risk of cancer in humans: An epidemiologic perspective. *Cytogenet. Genome Res. 104*, 376–382.
[73] Albertson, D. G., Collins C., McCormick, F., and Gray, J. W. (2003) Chromosome aberrations in solid tumors. *Nature Genet. 34*, 369–376.
[74] Bender, M. A., Griggs, H. G., and Walker, P. L. (1973) Mechanisms of chromosomal aberration production. I. Aberration induction by ultraviolet light. *Mutat. Res. 20*, 387–402.
[75] Bender, M. A., Bedford, J. S., and Mitchell, J. B. (1973) Mechanisms of chromosomal aberration production. II. Aberrations induced by 5-bromodeoxyuridine and visible light. *Mutat. Res. 20*, 403–416.
[76] Ishidate, Jr., M., Harnois, M. C., and Sofuni, T. (1988) A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell culture. *Mutat. Res. 195*, 151–213.
[77] Mitchell, A. D., Auletta, A.E., Clive, D., Kirby, P. E., Moore, M. M., and Myhr, B. C. (1997) The L5178Y/tk+/− mouse lymphoma specific gene and chromosomal mutation assay a phase III report of the U. S. Environmental Protection Agency Gene-Tox Progam. *Mutat. Res. 394*, 177–303.
[78] Newbold, R. R. (2004) Lessons learned from perinatal exposure to diethylstilbestrol. *Toxicol. Appl. Pharmacol. 199*, 142–150.

[79] Desjardins, R., Fournier, M., Denizeau, F., and Krzystyniak, K. (1992) Immunosuppression by chronic exposure to N-nitrosodimethylamine (NDMA) in mice. *J. Toxicol. Environ. Health 37*, 351–361.

[80] Simeonova, P. P., and Luster, M. I. (2000) Mechanisms of arsenic carcinogenicity: Genetic or epigenetic mechanisms. *J. Environ. Pathol. Toxicol. Oncol. 19*, 281–286.

[81] Waalkes, M. P. (2003) Cadmium carcinogenesis. *Mutat. Res. 533*, 107–120.

[82] Evans, G. W., and Pouchnik, D. J. (1993) Composition and biological activity of chromium-pyridine carboxylate complexes. *J. Inorg. Biochem. 49*, 177–187.

[83] Broadhurst, C. L., Schmidt, W. F., Reeves III, J. B., Polansky, M. M., Gautschi, K., and Anderson, R. A. (1997) Characterization and structure by NMR and FTIR spectroscopy, and molecular modeling of chromium(III) picolinate and nicotinate complexes utilized for nutritional supplementation. *J. Inorg. Biochem. 66*, 119–130.

[84] Lanca, S., Alves, A., Vieira, A. I., Barata, J., de Freitas, J., and de Carvalho, A. (2002) Chromium-induced toxic hepatitis. *Eur. J. Int. Med. 13*, 518–520.

[85] Stearns, D. M., Wise, J. P., Sr., Patierno, S. R., and Wetterhahn, K. E. (1995) Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. *FASEB J. 9*, 1643–1649.

[86] Bagchi, D., Stohs, S. J., Downs, B. W., Bagchi, M., and Preuss, H. G. (2002) Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology 180*, 5–22.

[87] Bianchi, V., Celotti, L., Lanfranchi, G., Majone, F., Marin, G., Montaldi, A., Sponza, G., Tamino, G., Venier, P., Zantedeschi, A., and Levis, A. G. (1983) Genetic effects of chromium compounds. *Mutat. Res. 117*, 279–300.

[88] Stearns, D. M., Silveira, S. M., Wolf, K. K., and Luke, A. M. (2002) Chromium(III) tris(picolinate is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. *Mutat. Res. 513*, 135–142.

[89] Yu, W., Sipowicz, M. A., Haines, D. C., Birely, L., Diwan, B. A., Riggs, C. W., Kasprzak, K. S., and Anderson, L. M. (1999) Preconception urethane or chromium(III) treatment of male mice: multiple neoplastic and non-neoplastic changes in offspring. *Toxicol. Appl. Pharmacol. 158*, 161–176.

[90] Cheng, R. Y., Alvord, W. G., Powell, D., Kasprzak, K. S., and Anderson, L. M. (2002) Microarray analysis of altered gene expression in the TM4 Sertoli-like cell line exposed to chromium(III) chloride. *Reprod. Toxicol. 16*, 223–236.

[91] Cheng, R. Y., Alvord, W. G., Powell, D., Kasprzak, K. S., and Anderson, L. M. (2002) Increased serum corticosterone and glucose in offspring of chromium(III)-treated male mice. *Environ. Health Perspect. 110*, 801–804.

[92] Cheng, R. Y., Hockman, T., Crawford, E., Anderson, L. M., and Shiao, Y. H. (2004) Epigenetic and gene expression changes related to transgenerational carcinogenesis. *Mol. Carcinog. 40*, 1–11.

[93] Jacquamet, L., Sun, Y., Hatfield, J., Gu, W., Cramer, S. P., Crowder, W., Lorigan, G. A., Vincent, J. B., and Latour, J.M. (2003) Characterization of chromodulin by X-ray absorption and electron paramagnetic resonance spectroscopies and magnetic susceptibility measurements. *J. Am. Chem. Soc., 125*, 774–780.

[94] Davis, C. M., Royer, A. C., and Vincent, J. B. (1994) Synthetic multinuclear chromium assembly activates insulin receptor kinase activity: Functional model for low-molecular-weight chromium-binding-substance. *Inorg. Chem. 36*, 5316–5320.

[95] Speetjens, J. K., Parand, A., Crowder, M. W., Vincent, J. B., and Woski, S. A. (1999) Low-molecular weight chromium-binding substance and biomimetic $[Cr_3O(O_2CCH_2CH_3)6(H_2O)_3]^+$ do not cleave DNA under physiologically-relevant conditions. *Polyhedron 18*, 2617–2624.

[96] Parand, A., Royer, A. C., Cantrell, T. R., Weitzel, M., Memon, N., Vincent, J. B., and Crowder, M. W. (1998) DNA nicking by a trinuclear chromium complex. *Inorg. Chim. Acta 268*, 211–219.
[97] Evans, G. W. Dietary supplementation with essential metal picolinates. U.S. Patent 4,315,927.
[98] Evans, G. W., and Pouchnik, D. J. (1993) Composition and biological activity of chromium-pyridine carboxylate complexes, *J. Inorg. Biochem. 49,* 177–187.
[99] Stearns, D. M., and Armstrong, W. H. (1992) Mononuclear and binuclear chromium picolinate complexes. *Inorg. Chem. 31*, 5178–5184.
[100] Armstrong, M. J., Gara, J. P., Gealy, R. 3rd, Greenwood, S. K., Hilliard, C. A., Laws, G. M., and Galloway, S.M. (2000) Induction of chromosome aberrations in vitro by phenolphthalein: Mechanistic studies. *Mutat. Res. 457*, 15–30.
[101] Gudi, R., Slesinski, R. S., Clarke, J. J., and San, R. H. (2005) Chromium picolinate does not produce chromosome damage in CHO cells. *Mutat. Res. 587*, 140–146.
[102] Speetjens, J. K., Collins, R. A., Vincent, J. B., and Woski, S. A. (1999) The nutritional supplement chromium(III) tris(picolinate) cleaves DNA. *Chem. Res. Toxicol. 12*, 483–487.
[103] Slesinski, R. S., Clarke, J. J., San, R. H. C., and Gudi, R. (2005) Lack of mutagenicity of chromium picolinate in the hypoxanthine phosphoribosyltransferase gene mutation assay in Chinese hamster ovary cells. *Mutat. Res. 585*, 86–95.
[104] Yu, Z. W., and Quinn, P. J. (1994) Dimethyl sulphoxide: A review of its applications in cell biology. *Biosci. Rep. 14*, 259–281.
[105] Hepburn, D. D. D., Xiao, J., Bindom, S., Vincent, J. B., and O'Donnell, J. (2003) Nutritional supplement chromium picolinate causes sterility and lethal mutations in *Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 100*, 3766–3771.
[106] Whittaker, P., Sans, R. H. C., Clarke, J. J., Seifried, H. E., and Dunkel, V. C. (2005) Mutagenicity of chromium picolinate and its components in Salmonella typhimurium and L5178Y mouse lymphoma cells. *Food Chem. Toxicol. 43*, 1619–1625.
[107] Coryell, V. H., and Stearns, D. M. (2006) Molecular analysis of *hprt* mutations induced by chromium picolinate in CHO AA8 cells, *Mutat. Res. 610*, 114–123.
[108] Xu, Z., Yu, Y., Schwartz J. L., Meltz M. L., and Hsie A. W. (1995) Molecular nature of spontaneous mutations at the hypoxanthine-guanine phosphoribosyltransferase (hprt) locus in Chinese hamster ovary cells. *Environ. Mol. Mutagen. 26*, 127–138.
[109] Jeffcoat, A. R. (2002) [$^{14}$C] Chromium picolinate monohydrate: Disposition and metabolism in rats and mice. Technical Report RTI/64U-6855/15P. Research Triangle Institute, Research Triangle Park, NC.
[110] Hepburn, D. D. D, and Vincent, J. B. (2002) In vivo distribution of chromium from chromium picolinate in rats and implications for the safety of the dietary supplement. *Chem. Res. Toxicol. 15*, 93–100.

*Chapter 11*

# Redox chemistry and biological activities of chromium(III) complexes

**Aviva Levina, Irma Mulyani, and Peter A. Lay**

Centre for Heavy Metals Research, and Centre for Structural Biology and Structural Chemistry, School of Chemistry, University of Sydney, NSW, 2006, Australia

## INTRODUCTION

As shown in the previous chapters of this book, major controversies surround the proposed role of Cr(III) as an essential micronutrient, as well as its use in nutritional supplements. Such controversies are partially caused by the lack of a clear understanding of the chemical mechanisms by which Cr(III) acts as an anti-diabetic agent. By contrast, chromium in its highest oxidation state, Cr(VI), is a well-recognized human carcinogen and a potent mutagenic and cytotoxic agent (for a review, see Refs [1–3]). A generally accepted mode of action of Cr(VI) (as chromate, $[CrO_4]^{2-}$) includes its efficient uptake by cells via anion channels and subsequent reactions with intracellular reductants (such as glutathione, ascorbate, or NAD(P)H), which leads to highly reactive and potentially DNA-damaging intermediates, including Cr(V) and Cr(IV) species and organic radicals [1]. Ultimately, these reactions lead to the formation of protein–Cr(III)–DNA and DNA–Cr(III)–DNA cross-links, which are highly stable and strongly disruptive to the transcription process, due to the kinetic inertness of Cr(III) [1–3].

While the efficient reduction of Cr(VI) to Cr(III) is universally accepted as a cause of Cr(VI) toxicity, the opposite process (oxidation of Cr(III) to Cr(VI)) was for a long time considered incompatible with biological conditions [4]. However, recent experimental data show that such an oxidation can be caused by biological oxidants ($H_2O_2$ or $ClO^-$) or by common enzymatic systems (glucose oxidase or xanthine oxidase in the presence of a corresponding substrate and $O_2$) at pH 7.4 and 37 °C [5]. This chapter discusses the consequences of Cr(III) oxidation to Cr(VI) (including the formation of Cr(IV) and Cr(V) intermediates) under biological conditions for the mechanisms of both beneficial and adverse actions of Cr(III)-containing nutritional supplements. This discussion is preceded by a brief overview of the main pathways for the formation of biological oxidants and their roles in health and disease, including diabetes.

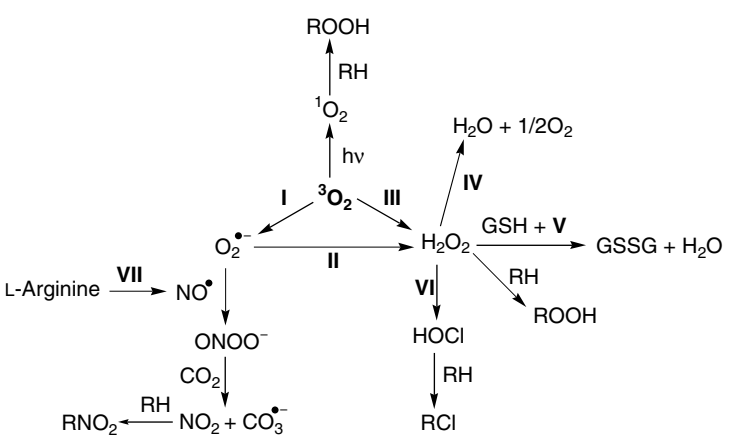
The Nutritional Biochemistry of Chromium(III)
***John B. Vincent (Editor)***
ISBN: 0-444-53071-1

## FORMATION AND ROLES OF STRONG OXIDANTS IN BIOLOGICAL SYSTEMS

A vast amount of literature on the chemistry and biology of oxygen and reactive oxygen species (ROS) is available (for reviews, see Refs [6–9]). Controlled oxidation of carbohydrates and lipids with $O_2$ to $CO_2$ and $H_2O$ provides the energy necessary to support life [8]. Yet, reactions of dioxygen in its ground (triplet, $^3O_2$) state with most biomolecules (having singlet ground states) are negligibly slow (spin-forbidden) under ambient conditions in the absence of catalysts [6]. The main pathways of $O_2$ activation in biological systems [7] are shown in a simplified form in Scheme 1.

Singlet oxygen ($^1O_2$, Scheme 1) is an activated form of dioxygen, formed by coupling of the two unpaired electrons in $^3O_2$, which removes the spin restriction for the direct reactions with organic compounds [6]. Singlet oxygen is usually formed in biological systems under UV irradiation in the presence of photosensitizers (organic dyes) [10]. It causes oxidative DNA damage, which can be either a trigger for radiation-induced cancers [11] or a basis of an anti-cancer treatment (photodynamic therapy) [12].

Dioxygen activation by redox enzymes (such as oxidases and oxygenases) is usually based on the direct or indirect reactions of $^3O_2$ with reduced forms of transition metal ions (Fe(II), Cu(I), Mn(II), or Mo(IV)) in the active centers of metalloenzymes [7]. The most common first step in such activation is the one-electron reduction of $O_2$ leading to superoxide radical ($O_2^{\bullet-}$ or $HO_2^{\bullet}$ in the protonated form) [6, 8]. This reaction is catalyzed by oxidases (**I** in Scheme 1), NAD(P)H oxidases and xanthine oxidases being dominant in mammalian organisms [9, 13, 14]. Reactions

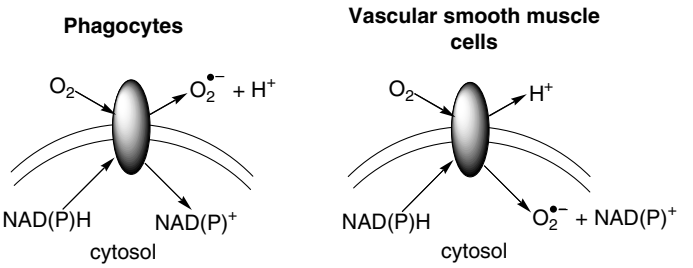



Scheme 1. Some major pathways for the formation and scavenging of strong oxidants in mammalian systems, based on the data from Refs [6–22]. Designations: ROOH, $RNO_2$, and RCl are the oxidized forms (peroxides, nitrates, or chlorides, respectively) of proteins or lipids (RH); GSH is glutathione ($\gamma$-Glu-Cys-Gly), and **I–VII** are the enzymatic systems (**I**, NAD(P)H oxidases or xanthine oxidase; **II**, superoxide dismutases; **III**, xanthine oxidase or monoamine oxidase; **IV**, catalase; **V**, glutathione peroxidase; **VI**, myeloperoxidase + $Cl^-$; and **VII**, nitric oxide synthases).

of $O_2^{\bullet-}$ with superoxide dismutases (SOD, **II** in Scheme 1) lead to the formation of hydrogen peroxide ($H_2O_2$), which is a stronger oxidant than $O_2^{\bullet-}$ [6, 7]. Some enzymes (**III** in Scheme 1), such as xanthine oxidases or amine oxidases in mammals [15, 16], as well as glucose oxidases in bacteria [17] and ascorbate oxidases in plants [18], are also capable of a direct two-electron reduction of $O_2$ with the formation of $H_2O_2$. In the past, it was often claimed that $H_2O_2$ could be converted by biological systems into the hydroxyl radical ($^{\bullet}OH$), which is an extremely powerful oxidant [6, 7], but most recent evidence does not support this suggestion (vide infra) [19].

Reactions of $^1O_2$, $O_2^{\bullet-}$, or $H_2O_2$ in biological systems can lead either to stable products ($H_2O$ or oxygen-containing functional groups of organic compounds) or to secondary oxidizing species [9]. The first pathway, called scavenging, is best known for $H_2O_2$ and includes the reactions with catalases (**IV** in Scheme 1) or peroxidases (e.g., glutathione peroxidase, **V** in Scheme 1) [7]. An example of the second pathway is the reaction of $H_2O_2$ with $Cl^-$, catalyzed by myeloperoxidase (**VI** in Scheme 1), which produces a strong oxidant, hypochlorite ($ClO^-$ or HOCl in the protonated form) [7]. Reactions of HOCl with biomolecules (RH in Scheme 1) lead to the formation of their chlorinated derivatives (RCl in Scheme 1), such as chloramines [7, 9]. Another example is the reaction of $O_2^{\bullet-}$ with nitric oxide ($NO^{\bullet}$), leading to peroxynitrite ($ONOO^-$), Scheme 1. This reaction is extremely fast since both the reagents are free radicals [9]. Nitric oxide, which is a crucial messenger molecule in the cardiovascular system, is produced by the oxidation of L-arginine that is catalyzed by NO-synthases (**VII** in Scheme 1) [20]. Most of the formed peroxynitrite probably reacts with $CO_2$, leading to the secondary radicals, $NO_2^{\bullet}$ and $CO_3^{\bullet-}$ (Scheme 1), and then to nitrated derivatives of biomolecules ($RNO_2$ in Scheme 1), such as nitrotyrosine [21, 22]. Reactions of $^1O_2$ or $H_2O_2$ (either spontaneous or catalyzed by enzymes such as lipoxygenases) with lipids or proteins lead to the formation of the corresponding peroxides (ROOH in Scheme 1) [7]. Further reactions of ROOH, which lead to the progressive formation of free radicals through chain reactions, are implicated as a major cause of a number of degenerative human diseases, as well as ageing [7, 9, 23]. Biological roles of other types of modifications in biological macromolecules, such as chlorination or nitration, are yet to be established [9].

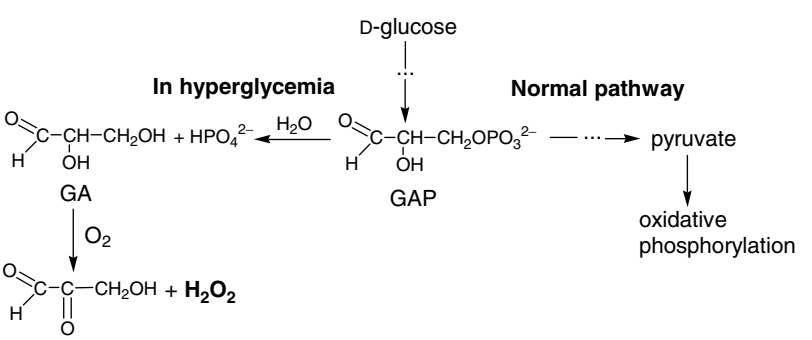
Biological roles of ROS (such as $O_2^{\bullet-}$ and $H_2O_2$) are determined by the mode of their production in cells [9]. For example, NAD(P)H oxidases, located in the cell membrane, are capable of releasing $O_2^{\bullet-}$ either into the extracellular fluid (in phagocytes) or into the cytoplasm (e.g., in vascular smooth muscle cells), as shown in Scheme 2 [13]. Release of $O_2^{\bullet-}$, $H_2O_2$, and HOCl into extracellular fluid by phagocytes is one of the major mechanisms of antimicrobial defense [7]. In recent years, the role of intracellular $O_2^{\bullet-}$ and $H_2O_2$ as important messenger molecules in cell signaling has emerged [9, 24, 25]. Concentrations of oxidizing species within cells are tightly regulated by scavenging enzymes (e.g., **II**, **IV**, and **V** in Scheme 1) and low-molecular-weight reductants (such as glutathione, ascorbate, or tocopherols) [7, 9]. Excessive accumulation of such species in cells ($>1\ \mu M\ H_2O_2$) triggers cell death via apoptosis [24, 25]. By contrast, extracellular fluids are largely devoid of ROS-scavenging enzymes, although there is some evidence for the existence of extracellular SOD and peroxidases [9]. Concentrations of non-enzymatic antioxidants (such as ascorbate or selenoproteins) in these fluids can vary




Scheme 2. Extra- and intracellular release of superoxide radicals by trans-membrane enzymes, NAD(P)H oxidases, based on the data from Ref. [13].

widely depending on the nutritional status and the presence of inflammatory conditions (leading to the release of strong oxidants by phagocytes, see above) [7, 9].

An overview of the main routes for the formation and scavenging of ROS in biological systems (Schemes 1 and 2) leads to a definition of oxidative stress as an imbalance between oxidants and antioxidants in favor of the former, potentially leading to damage [9, 26]. One of the major causes of chronic oxidative stress in humans is the altered glucose metabolism caused by diabetes (both type I and type II) (for a review, see Ref. [27]). One of the proposed mechanisms for the excessive formation of strong oxidants in diabetes is shown in Scheme 3 [27, 28]. Glucose overload leads to the formation of an unwanted by-product of glycolysis, glyceraldehyde, which is a strong reductant and reacts with $O_2$ at a significant rate and leads to the production of $H_2O_2$ (Scheme 3). In turn, excess $H_2O_2$ can cause oxidative damage of insulin-producing pancreatic cells (which have relatively poor antioxidant defenses compared with other types of cells), which leads to aggravation of glucose imbalance [27]. Chronic oxidative stress is considered as a major cause of the common complications of diabetes, including cardiovascular disease, retinopathy, and kidney failure [27], as well as of other insulin-related disorders, such as Alzheimer's disease [29]. A possible link between the




Scheme 3. One of the possible pathways for the excessive formation of $H_2O_2$ in diabetic patients, based on the data from Refs [27] and [28]. Designations: GAP = glyceraldehyde-3-phosphate; GA = glyceraldehyde.

anti-diabetic activity of Cr(III) complexes and oxidative stress conditions is explained in the Section 'Oxidative hypothesis of Cr(III) biological activity'.

## OXIDATION OF Cr(III) COMPLEXES UNDER PHYSIOLOGICAL CONDITIONS

Reactions of Cr(III) compounds with strong oxidants were studied by chemists for decades, but biological implications of these reactions have been recognized only recently [5, 30, 31]. Typical conditions for chemical oxidations of Cr(III) to Cr(VI) include the reactions with $H_2O_2$ or hexacyanoferrate ($[Fe(CN)_6]^{3-}$) in alkaline aqueous solutions (pH > 10) [32–34] or the reactions with permanganate ($[MnO_4]^-$), periodate ($[IO_4]^-$), or *N*-bromosuccinimide in weakly acidic or neutral aqueous solutions (pH 3–7) [4, 35, 36]. Oxidation of Cr(III) to Cr(VI) with $H_2O_2$ in alkaline media (pH > 12, Eq. 1), followed by spectrophotometric determination of Cr(VI), is a common method for the determination of Cr content in industrial and environmental samples [32, 33]. Oxidations of some Cr(III) complexes (with non-biological ligands) to Cr(VI) by $H_2O_2$ or $ClO^-$ at pH ~ 7 have also been reported [37, 38]. It should be noted that the redox potential (or oxidizing power, see Ref. [7] for details) of $H_2O_2$ increases with increasing pH values, while that of Cr(VI) decreases. In acidic media (pH < 5), Cr(VI) acts as an oxidant toward $H_2O_2$, and the reactions lead to the formation of $O_2$ and Cr(III) complexes (e.g., Eq. 2) [39]. In neutral media (pH 6–8), the redox potentials of Cr(VI) and $H_2O_2$ are close, and the reactions proceed through complicated mechanisms, which leads to disproportionation of $H_2O_2$ (Eq. 3) and to the formation of transient Cr(VI) and Cr(V) peroxo species (but not stable Cr(III) complexes) [40–42].

$$2[Cr^{III}(OH)_6]^{3-} + 3H_2O_2 \rightarrow 2[Cr^{VI}O_4]^{2-} + 2OH^- + 8H_2O \text{ (alkaline medium)} \quad (1)$$

$$2[HCr^{VI}O_4]^- + 3H_2O_2 + H_2O + 8H^+ \rightarrow 2[Cr^{III}(OH_2)_6]^{3+} + 3O_2 \text{ (acidic medium)} \quad (2)$$

$$2H_2O_2 \; (+ \; [Cr^{VI}O_4]^{2-}) \rightarrow O_2 + 2H_2O \; (+ \; [Cr^{VI}O_4]^{2-}) \text{ (neutral medium)} \quad (3)$$

Oxidation of Cr(III) and reduction of Cr(VI) in neutral aqueous solutions have been studied extensively in environmental chemistry (for reviews, see Refs [43] and [44]). Natural waters contain comparable amounts of dissolved Cr(III) and Cr(VI) (in the 1 nM–1 μM concentration range) [45–47], while sediments contain mostly insoluble Cr(III) hydroxo complexes [43]. Oxidation of Cr(III) to Cr(VI) in natural waters and soils is thought to be carried out mainly by Mn(IV) and Mn(III) oxides [48, 49], but a significant role for $H_2O_2$ (formed from $O_2$ in light-catalyzed reactions), particularly in sea waters, has also been proposed [50–52]. Reduction of Cr(VI) to Cr(III), which is more prevalent in fresh waters and soils compared with seawater, is carried out by Fe(II)- and sulfide-containing minerals, dissolved organic matter, and bacteria [44, 53], but a role for bacteria in the re-oxidation of Cr(III) to Cr(VI) has also been proposed [54].

Recent studies [5] have confirmed the propensity of $H_2O_2$, $ClO^-$ or $H_2O_2$-producing enzymatic systems (based on glucose oxidase or xanthine oxidase) to oxidize biologically relevant Cr(III) complexes to Cr(VI) under physiological conditions. For example, the reaction of the trinuclear Cr(III) propionate complex ($[Cr_3O(OCOEt)_6(OH_2)_3]^+$, 0.10 mM) with $H_2O_2$ (1.0 mM) in undiluted fetal bovine

serum produced Cr(VI) ($8 \pm 2\,\mu M$) within 1 hour at 37 °C [5]. The same Cr(III) complex can be oxidized to Cr(VI) by sub-millimolar concentrations of *tert*-butyl hydroperoxide ($^t$BuOOH, a commonly used model of peroxides of proteins or lipids) [55] in the presence of high concentrations of catalase (i.e., under the conditions when the oxidation by $H_2O_2$ is completely inhibited) [56]. This Cr(III) complex has been chosen for oxidation studies [5] due to its known biological activity [57–60] and proposed structural similarity to a purported natural Cr(III)-containing factor (chromodulin, see Chapter 7 of this book) [61, 62]. Other Cr(III) complexes, such as hydrolyzed $CrCl_3$ (a mixture of polynuclear aqua-hydroxo complexes, vide infra) [63] or Cr(III) picolinate ($[Cr(pic)_3]$, a widely used but controversial nutritional supplement, see Chapters 4, 5, 8, and 9 of this book) could be oxidized to Cr(VI) by $H_2O_2$ in neutral aqueous solutions, although less efficiently than $[Cr_3O(OCOEt)_6(OH_2)_3]^+$ [5]. Chromium(III) complexes with serum proteins, such as albumin or transferrin could also be oxidized to Cr(VI) under similar conditions [56].

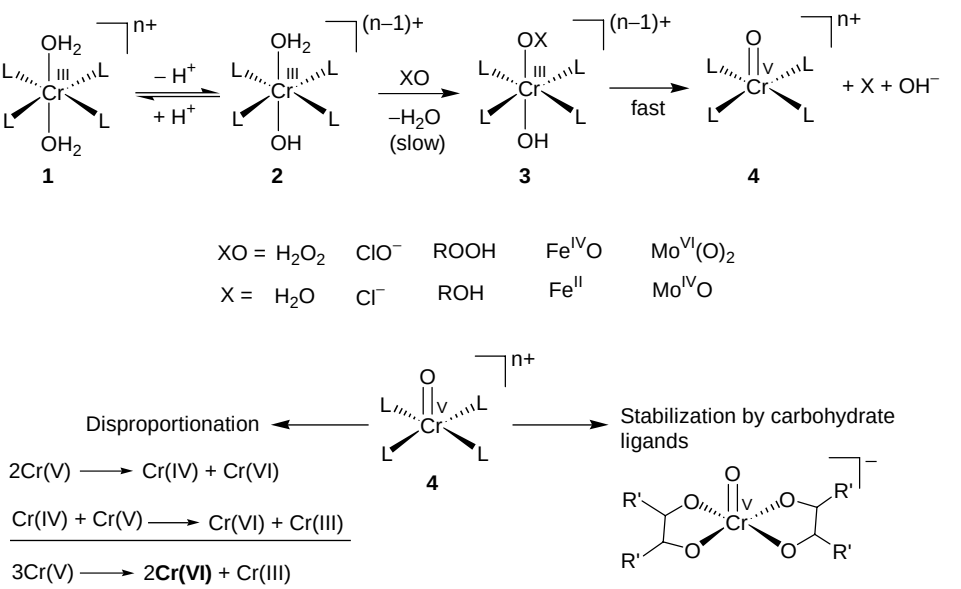
In a related issue, small amounts of Cr(VI) have been detected in leather samples treated by Cr(III) tanning salts [64]. The role of Cr(III) in leather tanning is the formation of cross-links between collagen molecules, which makes the leather resistant to bacterial degradation [65]. A likely reason for the formation of Cr(VI) in tanned leather is the oxidation of Cr(III)-collagen complexes by lipid peroxides (ROOH in Schemes 1 and 4) that originate from oils used in the leather processing [64]. Some authors suggested that such oxidation can only occur during the contact of Cr(III)-treated leather with aqueous buffer solutions ($pH \geq 7$), used in the standard analytical procedure for the determination of Cr(VI) in leather [66].

Formation of relatively stable Cr(V) intermediates during the reduction of Cr(VI) in biological or biomimetic systems has been studied in great detail (for a review, see Ref. [1]). Such intermediates have been implicated as the main, if not ultimate, reactive species in Cr(VI)-induced genotoxicity and carcinogenicity [31, 67]. Electron paramagnetic resonance (EPR) spectroscopy is used as a sensitive and selective method for the detection of Cr(V) intermediates formed during the reduction of Cr(VI) in vitro or in vivo [1]. Oxidation of Cr(III) complexes with certain types of ligands, such as aromatic imines [68, 69] or oligopeptides [70, 71], also leads to the formation of Cr(V) intermediates, which can be detected by EPR spectroscopy and in some cases isolated in a pure form and fully characterized. For instance, oxidation of *cis*-$[Cr^{III}(phen)_2(OH_2)_2]^{3+}$ (phen = 1,10-phenanthroline) by $ClO^-$ in neutral or weakly acidic aqueous solutions leads to quantitative formation of *cis*-$[Cr^{V}(O)_2(phen)_2]^+$ [69]. Oxidations of *trans*-$[Cr^{III}(salen)(OH_2)_2]^+$ ($salenH_2 = N,N'$-ethylenebis(salicylideneimine)) by iodosobenzene (PhIO) in non-aqueous media or by $PbO_2$ in aqueous solutions (pH 3–7) led to the formation of $[Cr^{V}O(salen)]^+$ [68, 72]. A Cr(III) hydroperoxo complex $[Cr^{III}(cyclam)(OOH)(OH_2)]^{2+}$ (cyclam = 1,4,8,11-tetraazacyclotetradecane) spontaneously converts into $[Cr^{V}O(cyclam)]^{3+}$ in neutral aqueous solutions [73, 74].

All the well-characterized Cr(V) complexes generated by the oxidation of Cr(III) precursors (including *cis*-$[Cr^{V}(O)_2(phen)_2]^+$, $[Cr^{V}O(salen)]^+$ and $[Cr^{V}O(cyclam)]^{3+}$) induce oxidative damage in isolated DNA in the absence of added $H_2O_2$ or other activators [72, 75, 76]. Importantly, the ability of some Cr(III) complexes, such as *cis*-$[Cr^{III}(phen)_2(OH_2)_2]^{3+}$ and *trans*-$[Cr^{III}(salen)(OH_2)_2]^+$, to be oxidized into the corresponding Cr(V) species under mild conditions, correlates with their ability to induce mutations (in the presence of $O_2$) in the strains of *Salmonella typhimurium* that are

sensitive to oxidative damage [72, 77]. By contrast, Cr(III) complexes that do not form stable Cr(V) species upon oxidation are non-mutagenic under these conditions [1, 72, 77]. Possible genotoxicity mechanisms of Cr(III) complexes and their oxidation products are discussed in the Section "Damage to DNA and proteins".

A general mechanism of Cr(III) oxidation to Cr(V) and Cr(VI) species has been proposed (Scheme 4) [31]. All biologically relevant Cr(III) compounds are six-coordinate octahedral complexes, in which the Cr(III) ions are surrounded by six donor atoms from organic or inorganic ligand molecules [63]. Most Cr(III) complexes capable of oxidation under mild conditions contain at least two aqua ligands (e.g., *cis*-$[Cr(phen)_2(OH_2)_2]^{3+}$, *trans*-$[Cr(salen)(OH_2)_2]^{+}$, or $[Cr_3O(OCOEt)_6(OH_2)_3]^{+}$) [5, 30, 72]. Such complexes are designated by a general structure $[CrL_4(OH_2)_2]^{n+}$ (**1** in Scheme 4), where L represents any monodentate or polydentate ligands (polydentate or chelating ligands are those containing two or more binding sites). Since the rates of oxidation of Cr(III) complexes in aqueous solutions generally increase with increasing pH values [35–37], deprotonation of **1** leading to an aqua-hydroxo complex (**2** in Scheme 4) is likely to occur prior to the oxidation [31], followed by the formation of a Cr(III)-oxidant complex, **3** (Scheme 4). Possible oxidants (Scheme 4) [31] include $H_2O_2$, $ClO^-$, organic peroxides (ROOH), and oxidized forms of metalloenzymes, such as the $Fe^{IV}O$ centers in cytochromes P450 [78] or $Mo^{VI}(O)_2$ centers in oxotransferases [79]. At present, there is no direct

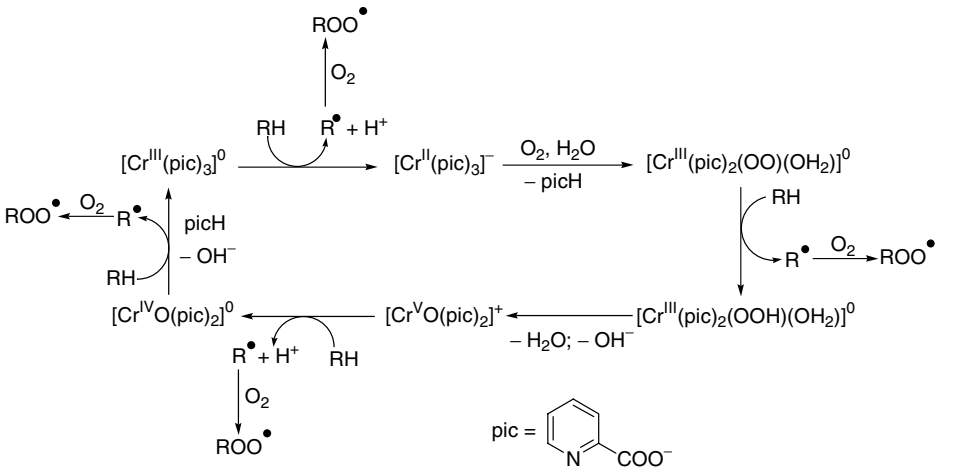



Scheme 4. Proposed mechanism of oxidation of Cr(III) complexes by biological oxo-transfer reagents (XO), based on the data from Ref. [31]. Charges of the complexes can vary depending on the nature of the ligands (L). Designations of the oxidants (XO): ROOH are peroxides of proteins or lipids; $Fe^{IV}O$ are the active centers of cytochrome P450 and related oxidases; and $Mo^{VI}(O)_2$ are the active centers of oxo-transferases.

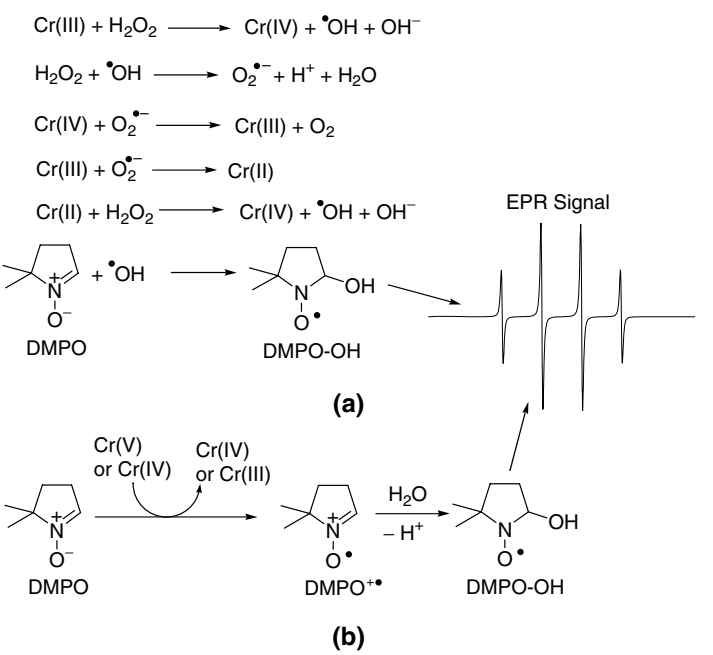
evidence for the formation of Cr(III)-oxidant complexes such as **3**, as rates of their formation are likely to be much lower than the rates of intramolecular electron-transfer reactions with the formation of Cr(V) oxo complexes (**4** in Scheme 4) [31]. Indirect evidence for the formation of **3** is the observation of a Cr(III) hydroperoxo complex ($[Cr^{III}(cyclam)(OOH)(OH_2)]^{2+}$, which corresponds to **3** with X = OH) as a product of one-electron reduction of a peroxo complex, $[Cr^{III}(cyclam)(OO)(OH_2)]^{2+}$ (which in turn forms in the reaction of the corresponding Cr(II) complex with $O_2$) [73, 76]. Formation of Cr(V) complexes **4** by intramolecular electron-transfer reactions in the Cr(III)-oxidant complexes **3** (Scheme 4) is supported by isotopic ($^{18}O$) labelling studies, which show that the oxo group in $[Cr^{V}O(salen)]^{+}$ originates from the oxidant (PhIO), rather than from the aqua ligands in the Cr(III) precursor complex [68]. Most of the known Cr(V) complexes have lifetimes of seconds or minutes under physiological conditions [1, 31] and are likely to undergo two main types of reactions (shown in Scheme 4): disproportionation with the formation of Cr(VI) and Cr(III) species [80] or ligand-exchange reactions with carbohydrates (including glycoproteins), which are known as excellent stabilizing ligands for Cr(V) at physiologically relevant concentrations and pH values [81].

Reactions of oxidants (such as $H_2O_2$) with Cr(III) complexes that do not contain aqua ligands (such as $[Cr(pic)_3]$) are slow [5] and are likely to involve hydrolysis (with the formation of more labile Cr(III) species such as $[Cr(pic)_2(OH)(OH_2)]$) as the first step [31]. It was also suggested that $[Cr(pic)_3]$ and other Cr(III) complexes with aromatic ligands (e.g., phen or salen) can be reduced to their Cr(II) analogs under physiological conditions by strong biological reductants such as ascorbate [82, 83]. No direct evidence for the formation of Cr(II) species in these reactions has been obtained as yet due to the extremely low stability of Cr(II) complexes in neutral aqueous solutions, particularly in the presence of $O_2$ [31]. Evidence for the formation of strongly oxidizing species in the $[Cr(pic)_3]$ + reductant + $O_2$ system is provided by the oxidative DNA cleavage in vitro, caused by this system (vide infra) [83, 84]. Possible mechanisms involved in $O_2$ activation by the $[Cr(pic)_3]$ + reductant system are shown in Scheme 5 [1, 83]. Although no reactive Cr intermediates have been observed directly in this system [83], most of the redox reactions proposed in Scheme 5 are based on detailed studies of similar reactions of Cr(II) complexes with other types of ligands [73, 74, 76]. In addition, detectable amounts of Cr(V)- and Cr(IV)-picolinato complexes have been generated by other methods [85, 86]. According to Scheme 5, the $[Cr(pic)_3]$ complex is not spent in the overall reaction, that is it acts as a catalyst for the reactions of reductants (RH in Scheme 5) with $O_2$, leading to peroxyl radicals ($ROO^{\bullet}$ in Scheme 5). These radicals, as well as the transient Cr(V)- and Cr(IV)-picolinato complexes (Scheme 5), are the likely reactive species in oxidative DNA cleavage (vide infra) [1]. Thus, in a seemingly paradoxical way, the initial reduction of Cr(III) to Cr(II) can lead, through the activation of $O_2$, to the formation of strongly oxidizing species, including Cr(IV) and Cr(V) complexes (Scheme 5).

Formation of strongly oxidizing species during the reactions of Cr(III) complexes with $H_2O_2$ or lipid hydroperoxides has been studied using spin-trap techniques [87, 88], but the results of such studies have to be interpreted with caution [1]. Observation of a characteristic four-line EPR signal due to DMPO-OH (Scheme 6, DMPO = 2,2-dimethylpyrrolidine-*N*-oxide) was attributed to the formation of $^{\bullet}OH$ radicals, as shown in Scheme 6a [87, 88]. However, the same signal can be generated via the direct oxidation of DMPO by Cr(V/IV) complexes (Scheme 6b) that are formed during the reactions




Scheme 5. Possible redox reactions of Cr(III) picolinato complexes (or other Cr(III) complexes with strong chelating ligands) in the presence of strong reductants and $O_2$, based on the data from Refs [1] and [83]. Designations: RH is a strong reductant such as ascorbate or dithiothreitol (Ref. [83]).




Scheme 6. Alternative mechanisms (based on data from Refs [1] and [87–89]) for the reactions of a spin trap, DMPO, with the oxidation products of Cr(III), leading to a characteristic four-line EPR signal of DMPO-OH.

of Cr(III) complexes with peroxides (Scheme 4) [1, 89]. Thus, observation of DMPO oxidation products by EPR spectroscopy points to the presence of strong oxidants in the reaction system, but it does not establish the nature of these oxidants. Although the available experimental data are insufficient to establish the nature of oxidants with certainty, there is a direct analogy with the Fe(II) + $H_2O_2$ or Fe(II) + reductant + $O_2$ systems (Fenton reactions) [90]. Decades of extensive studies have not settled the controversy over the nature of reactive species in Fenton reactions [91], but growing evidence points to the formation of Fe(IV) and Fe(V) oxo complexes as the major oxidants rather than $^{\bullet}$OH radicals [19]. Other observations pointing to the formation of strongly oxidizing species during the reactions of Cr(III) complexes with peroxides include the following: (i) catalysis by Cr(III) of the oxidation of fluorescent dyes with $H_2O_2$, used as an analytical method for the detection of Cr(III) in environmental samples [92] and (ii) oxidative DNA cleavage in vitro by the Cr(III) + $H_2O_2$ systems (vide infra).

## BIOLOGICAL CONSEQUENCES OF Cr(III) OXIDATION

### Is Cr(III) an antioxidant or a pro-oxidant?

One of the controversies surrounding the use of Cr(III)-containing nutritional supplements concerns the proposed roles of such supplements as antioxidants that reduce the diabetes-related oxidative stress [93–96], or pro-oxidants that promote the oxidative stress through the formation of ROS [97–99]. Typical reported examples of the both actions of Cr(III) are described below.

In an early report on the antioxidant action of Cr(III) [100], pre-treatment of mice with $CrCl_3$ (single injection of an aqueous solution, 5 mg kg$^{-1}$) was found to reduce the toxic effects of $CCl_4$ (including lipid peroxidation in the liver), which are thought to be related to the formation of strongly oxidizing $^{\bullet}CCl_3$ radicals. Additions of $CrCl_3$ (1–10 μM, alone or in combination with 17 β-estradiol) to the cell culture medium significantly reduced the levels of oxidized lipids and proteins in a human monocyte cell line treated with $H_2O_2$ or with excessive glucose concentrations [93, 101]. Synergistic action of grape seed extract and niacin-bound Cr(III) (2.2 mg kg$^{-1}$ for 10 weeks) in reducing the harmful effects of high-fat diets (including high levels of lipid peroxides in the blood plasma) in Syrian hamsters has been described [94]. Significant decreases in the plasma lipid peroxidation levels in type II diabetic patients after a course of Cr(III) supplementation were detected in two randomized, double-blind, and placebo-controlled human studies [95, 96]. In the first of these studies [95], a 6-week course of 0.40 mg Cr/day (as tris(2-pyrrolidine-5-carboxylato)chromium(III), a complex similar in chemical properties to [$Cr(pic)_3$]) was used for the patients having fasting blood glucose (FBG) levels of >8 mM. In the second study [96], Cr(III) was administered at 1.0 mg/day for 6 months in the form of Cr(III)-enriched yeast, and this study was the only one to compare the effects of Cr(III) supplementation at various blood glucose levels. The decreases in plasma lipid peroxidation levels, caused by Cr(III) supplementation, were more pronounced in severely hyperglycemic patients (FBG >8.5 mM) than in mildly hyperglycemic patients (FBG = 7.3–8.4 mM), while significant increases in lipid peroxidation were observed in euglycemic (non-diabetic) subjects (FBG = 4.7–5.3 mM) [96]. Notably, none of the studies [95, 96] detected significant changes in blood glucose levels following Cr(III) supplementation.

The ability of Cr(VI) to cause oxidative stress in exposed humans and in animal models is well-documented [1, 97, 102], but similar effects of Cr(III) were little known until recently. Levels of urinary lipid peroxidation in leather tanning workers (exposed to Cr(III) but not Cr(VI)) were significantly ($p < 0.01$) increased over the controls and only slightly lower than the levels of such peroxidation in stainless steel welders, exposed predominantly to Cr(VI) [103]. Oral administration of high doses of Cr(III) to rats (895 mg $kg^{-1}$, as aqueous $CrCl_3$) caused significant increases in several markers of oxidative stress, including urinary excretion of oxidized lipid metabolites, hepatic lipid peroxidation, and superoxide radical production [97]. Similar but stronger effects were caused by administration of lower doses of Cr(VI) (25 mg $kg^{-1}$ in water) [97]. Intravenous administration of $[Cr(pic)_3]$ to rats (0.090 μmol/day for 60 days) caused significant increases in the levels of urinary lipid oxidation products and 8-hydroxy-2′-deoxyguanosine (8-OHdG, a marker of oxidative DNA damage) [98]. Treatments of cultured murine macrophages with 10–50 μg $mL^{-1}$ of either $[Cr(pic)_3]$ or niacin-bound Cr(III) (a poorly characterized compound, probably a mixture of polynuclear Cr(III) carboxylato complexes) [104] caused increased DNA fragmentation and ROS production, but these effects were significantly more pronounced for $[Cr(pic)_3]$ [97]. Treatments of cultured human macrophages with Cr(III) (as $CrCl_3$, up to 250 ppm) caused the formation of oxidized proteins (detected by the presence of carbonyl groups) [99]. Finally, numerous in vitro studies showed the production of strongly oxidizing species, capable of causing DNA and protein damage, in the Cr(III) + $H_2O_2$ or Cr(III) + reductant + $O_2$ reaction systems (vide supra and vide infra).

The dual action of Cr(III) as an antioxidant or a pro-oxidant, described above, can be explained based on the redox reactions in Schemes 4 and 5. The reactions of Cr(III) complexes with lipid peroxides (ROOH in Scheme 4) are probably responsible for the abilities of these compounds to reduce the levels of lipid peroxidation [93–96, 100], but these reactions produce other strong oxidants such as Cr(V) species (Scheme 4). These species, as well as the peroxyl ($ROO^{\bullet}$) radicals formed in the redox cycling reactions of certain Cr(III) complexes (Scheme 5), are probably responsible for the increases in the oxidative stress markers caused by Cr(III) administration [97–99, 103]. Thus, Cr(III) complexes used as nutritional supplements are involved in a delicate balance between the oxidation and the reduction reactions in blood plasma, as shown most clearly by a change from a mild antioxidant effect of a prolonged treatment with Cr(III) in type II diabetic patients to a mild pro-oxidant effect of the same treatment protocol in healthy individuals [96]. The described controversy over the roles of Cr(III) in biological redox reactions does not support the suggestion [95, 105] that the antioxidant action of Cr(III) supplements may be a major reason for their anti-diabetic activities.

**Damage to DNA and proteins**

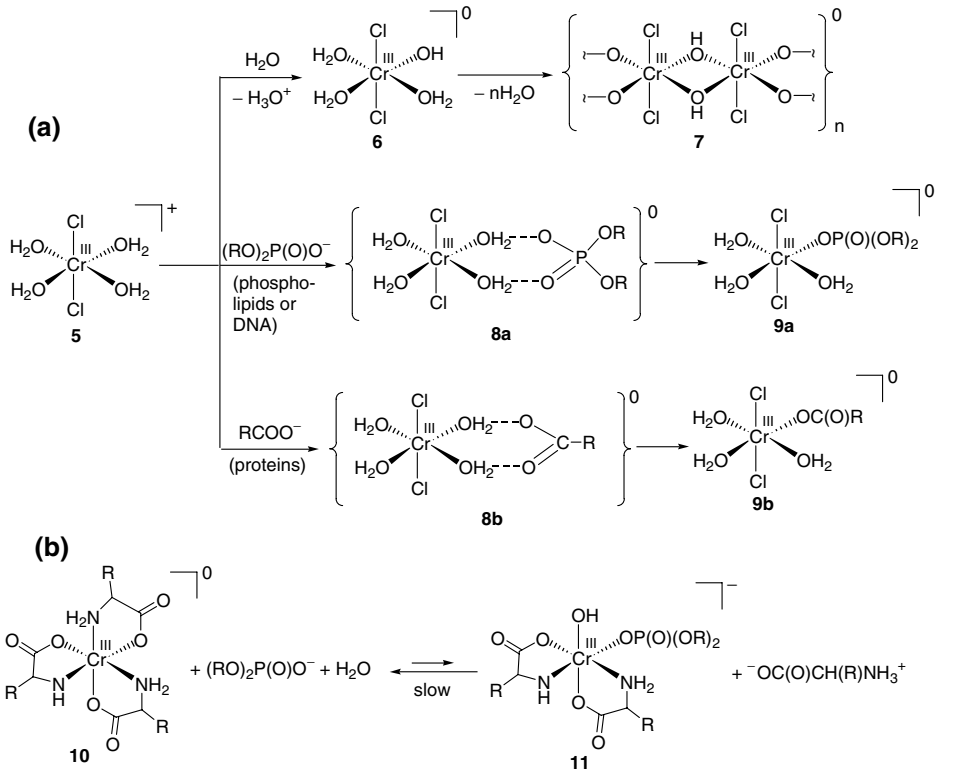
Currently, the main concern over Cr(III)-induced DNA damage is focused on the use of $[Cr(pic)_3]$ as a nutritional supplement (see Chapters 9 and 10 of this book). Several authors suggested that the deleterious actions of $[Cr(pic)_3]$ are entirely due to the picolinato ligand and that the use of alternative Cr(III) compounds, such as trinuclear Cr(III) propionate [58, 106], niacin-bound Cr(III) [94], or $[Cr^{III}L_3]$ complexes (where LH = histidine or phenylalanine) [107, 108], is safe. However, other studies have shown a genotoxic potential of Cr(III) compounds other than $[Cr(pic)_3]$. For instance,

a preconception exposure of male mice to aqueous $CrCl_3$ (1.0 mmol $kg^{-1}$ as a single intraperitoneal injection) did not cause an acute toxicity but led to multiple neoplastic transformations in the progeny [109]. Exposure to Cr(III) (as a mixture of Cr(III) aqua and hydroxo complexes) [63, 65] in leather tannery workers led to a pattern of DNA damage in lymphocytes, including DNA-protein cross-links and an increased incidence of micronuclei, which was similar to genotoxicity markers found in stainless steel welders exposed to Cr(VI)-containing fumes [103]. These results point to a similarity in the mechanisms of DNA damage, promoted by either Cr(III) or Cr(VI) compounds.

Over the last 20 years, many authors [110–113] claimed that Cr(III) is the ultimate genotoxic form of Cr based on the ability of aquated $CrCl_3$ (mainly *trans*-$[Cr^{III}Cl_2(OH_2)_4]^+$) or $Cr(NO_3)_3$ ($[Cr^{III}(OH_2)_6]^{3+}$) [63] to bind to isolated DNA (causing changes in DNA conformation), and on the absence of such binding for Cr(VI). Others [114] found that DNA lesions produced by $CrCl_3$ were non-mutagenic in mammalian cell assays. Indeed, the use of $CrCl_3$ or $Cr(NO_3)_3$ as "representative" Cr(III) compounds is the most persistent mistake in studies of biological roles of Cr(III), since it does not take into account their incompatibility with neutral aqueous solutions [1]. Some of the likely transformations of these compounds in biological media are illustrated in Scheme 7a (for a review, see Ref. [63]).

Dissolution of $CrCl_3 \cdot 6H_2O$ (*trans*-$[Cr^{III}Cl_2(OH_2)_4]^+$, **5** in Scheme 7a) in water leads to rapid deprotonation of **5** (which is a stronger acid than $H_2O$) with the formation of an uncharged species **6**, which is then gradually converted into a mixture of water-insoluble polynuclear hydroxo complexes (**7** in Scheme 7a) [63]. Biological media contain many types of organic bases, including deprotonated phosphato groups of phospholipids or DNA and carboxylato residues of proteins ($(RO)_2P(O)O^-$ and $RCOO^-$, respectively, in Scheme 7a), that will react with **5** more readily than $H_2O$. These reactions proceed through an initial rapid formation of ion pairs between the positively charged species **5** and the negatively charged functional groups of biomolecules (**8a, b** in Scheme 7a), followed by slower formation of covalently bound Cr(III)-biomolecule adducts (**9a, b** in Scheme 7a) [115]. Binding of Cr(III) to the phospholipids of cellular or nuclear membranes, caused by the additions of freshly prepared aqueous solutions of $CrCl_3$, is probably responsible for the reported "efficient uptake" of this compound by isolated cell nuclei [116] or by whole cells [114]. Pre-incubation of $CrCl_3$ with cell culture media, which probably leads to the formation of Cr(III) complexes with amino acids and proteins [1, 63], dramatically decreases the cellular binding of Cr(III) [56]. Generally, Cr(III) complexes are very slow to penetrate cell membranes, due to their octahedral structures and kinetic inertness [1, 30]. Nevertheless, a significant accumulation of Cr(III) in cell compartments, including the nuclei, can occur as a result of occupational exposure to Cr(III) [103] or a long-term excessive use of Cr(III)-containing nutritional supplements [117].

In a way that is similar to the processes described in Scheme 7a, stable cationic Cr(III) complexes with polypyridyl ligands, such as $[Cr(phen)_3]^{3+}$ and its analogs, strongly bind to isolated DNA in vitro due to the electrostatic attraction of the positively charged complex ions to the negatively charged DNA backbone [118]. Binding of these photo-sensitive Cr(III) complexes makes DNA susceptible to photo-induced oxidation by $O_2$, probably through the formation of singlet oxygen ($^1O_2$ in Scheme 1) [119]. These reactions are unlikely to have major implications in the in vivo systems, since: (i) highly




Scheme 7. Typical ligand-exchange reactions of Cr(III) complexes in aqueous solutions in the presence or absence of biological ligands, based on the data from Refs [1] and [63] (R are the residues of amino acids, proteins, phospholipids, or DNA).

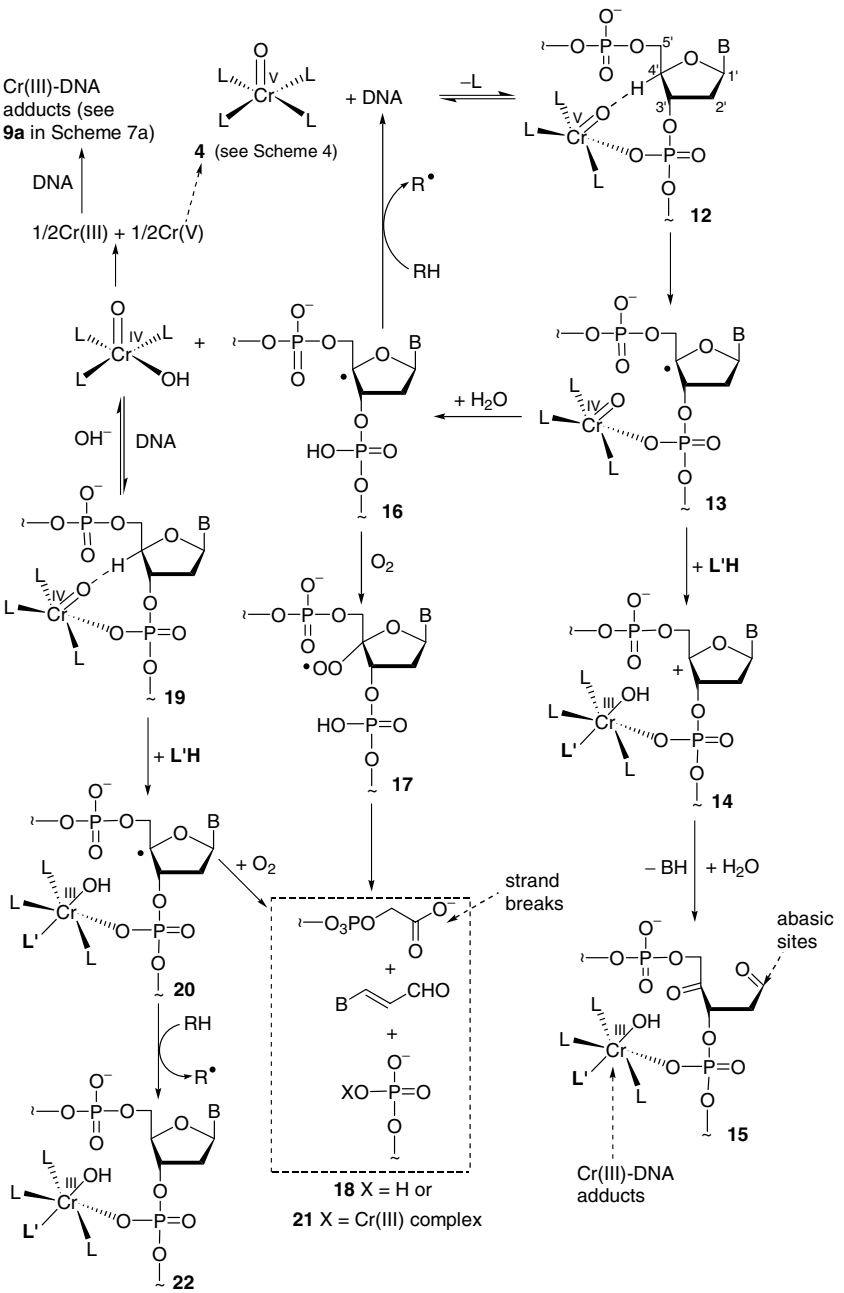
charged Cr(III) complexes will not penetrate cellular and nuclear membranes to a significant extent [1]; and (ii) electrostatic attraction of cationic Cr(III) complexes to DNA in cells will be greatly reduced due to the masking of the negative charge of DNA by positively charged histones in chromatin [120].

Although the exact chemical nature of Cr(III) complexes in biological systems remains unknown (see Chapters 1 and 2 of this book), stable uncharged Cr(III) complexes with amino acids or peptides (of a general structure **10**, Scheme 7b) are among the likely biological forms of Cr(III) [1]. Reactions of such complexes with DNA or other biological macromolecules (leading to mixed-ligand species such as **11** in Scheme 7b) are unfavorable for both thermodynamic and kinetic reasons (removal of a strong chelating ligand and kinetic inertness of the Cr(III) ion, respectively) [63]. The absence of a significant DNA binding for Cr(III) complexes with amino acids (such as cysteine) or peptides (such as glutathione) at pH $\geq$ 7 has been confirmed experimentally [114, 121, 122].

There are numerous reports on the oxidative DNA cleavage in vitro by $H_2O_2$ in the presence of Cr(III) complexes, including aquated $CrCl_3$ [123–129], trinuclear Cr(III)

oxo-carboxylates [106, 130], and Cr(III)-ascorbate and Cr(III)-glucose complexes [131]. The biological significance of such reactions is questionable due to the very low concentrations of $H_2O_2$ in cells, particularly in the vicinity of DNA (Section 'Formation and roles of strong oxidants in biological systems') [25]. In the absence of Cr(III) (or other transition metal ions such as Fe(III) or Cu(II)), $H_2O_2$ does not cause significant DNA damage [123, 130]. Hydroxyl ($^{\bullet}OH$) radicals are usually implicated as the main DNA-cleaving species in the Cr(III) + $H_2O_2$ systems, based on the observation of the DMPO-OH signals in EPR spectra (Scheme 6a) [123] or inhibition of DNA cleavage by "radical scavengers" such as mannitol or melatonin [124, 129]. However, these data are also consistent with Cr(V/IV) complexes being the main DNA-cleaving species in the Cr(III) + oxidant systems (Schemes 6b and 8, see below for details) [1]. No Cr(V/IV) species have been detected directly as yet under the conditions of the Cr(III) + $H_2O_2$ + DNA reactions [56, 130], probably due to the high reactivity and low steady-state concentrations of such species [1]. On the other hand, reactions of biologically relevant Cr(III)-oligopeptide complexes with mild oxidants (such as $PbO_2$ or $H_5IO_6$, mimicking the biological oxo-transfer agents listed in Scheme 4) in neutral aqueous solutions induced oxidative DNA cleavage and led to the detection of Cr(V) intermediates by EPR spectroscopy [70]. These data also show that the presence of $H_2O_2$ as a source of $^{\bullet}OH$ radicals (Scheme 6a) is not required for DNA cleavage that is induced by Cr(III) oxidation products, which supports the role of Cr(V/IV) intermediates in such reactions.

The propensity of Cr(V) and Cr(IV) compounds to induce oxidative DNA cleavage in the absence of added oxidants or reductants has been firmly established with the use of model Cr(V/IV) complexes, predominantly those with 2-hydroxycarboxylato ligands [75, 132–138]. These studies led to the proposal of a mechanism of Cr(V/IV)-induced DNA cleavage, shown in Scheme 8 [133, 137, 138]. Oxidation of Cr(III) complexes leads to the formation of Cr(V) oxo complexes **4** (Schemes 4 and 8), which can bind rapidly to the phosphate backbone of DNA to form Cr(V)–DNA intermediates (**12** in Scheme 8) [139]. Unlike for kinetically inert Cr(III) complexes, such as **11** in Scheme 7b, Cr(V) complexes, such as **4**, are kinetically labile, that is capable of rapid ligand-exchange reactions [1, 31]. Complex **12** then undergoes an intramolecular electron-transfer reaction with the formation of a Cr(IV) complex of a DNA radical (**13** in Scheme 8) [138]. The 4′ position of the deoxyribose ring has been proposed as the main site of DNA oxidation by Cr(V) 2-hydroxycarboxylato complexes, based on studies of DNA oxidation products [132]. An unstable intermediate, **13**, can undergo a second electron-transfer reaction to form a Cr(III) complex of a DNA cation (**14** in Scheme 8), which rapidly reacts with $H_2O$ to form a Cr(III)–DNA complex (stable because of the kinetic inertness of Cr(III)), which also possesses an abasic site (**15** in Scheme 8) [137]. Alternatively, dissociation of a Cr(IV) complex (which is kinetically labile like Cr(V)) from **13** can occur, which leads to a DNA radical, **16** (Scheme 8). Such radicals react with C–H bonds of most organic compounds, which leads to the regeneration of the original DNA molecule (Scheme 8) [137]. Such regeneration reactions explain the efficient inhibition of Cr(V)-induced DNA cleavage by common organic buffers or solvents [137], as well as by the purported $^{\bullet}OH$ scavengers such as melatonin or mannitol [124, 129] (the latter also decreases the reactivity of Cr(V) species by forming stable Cr(V) complexes) [1]. Alternatively, in the presence of $O_2$, DNA radicals **16** can be converted to peroxyl




Scheme 8. Main pathways of DNA damage induced by Cr(III) oxidation products, based on the data from Refs [137] and [138]. Designations: RH is any organic substrate capable of H• abstraction reaction; B is a nucleic base; and L and L′ are the ligands (charges of the complexes are omitted for clarity).

radicals **17**, which can then react with $H_2O$ to form DNA strand-cleavage products **18** (Scheme 8) [137]. Dissociation of a Cr(IV) species from **13** can lead to the formation of a new Cr(IV)–DNA intermediate, **19**, which can then undergo similar reactions to those of a Cr(V)–DNA intermediate, **12** (Scheme 8). These reactions lead, through the formation of a Cr(III) complex of a DNA radical, **20**, either to DNA strand cleavage products, **21** (with a Cr(III) complex attached to one of the fragments) or to a Cr(III) complex of intact DNA, **22**, formed as a result of a radical regeneration reaction (Scheme 8) [137].

Scheme 8 illustrates possible mechanisms for the formation of the main types of Cr(V/IV)-induced DNA lesions, which include Cr(III)–DNA adducts (**15**, **21** or **22**), single-strand breaks (**18** or **21**), and abasic sites (**15**) [1, 67], but it does not exhaust all the available reaction pathways. For instance, the use of other types of Cr(V/IV) complexes (rather than 2-hydroxycarboxylates) [133, 137, 138] can lead to different oxidation products, including those originating from the hydrogen abstraction at the 1′ position of deoxyribose ring [134], or from the oxidation of guanine bases [75, 135]. Addition of $H_2O_2$ to the Cr(V) + DNA reaction mixtures led to a significant increase in the extent of DNA cleavage and to a different distribution of cleavage products [133], probably due to the formation of highly reactive Cr(V/IV) peroxo complexes [133, 140, 141]. Similarly, formation of Cr(V/IV) peroxo intermediates during the Cr(III) + $H_2O_2$ + DNA reactions may explain the detection of double-strand break products and oxidized nucleic bases (including 8-OHdG), which is not explained by the mechanism proposed in Scheme 8 and is usually attributed to the action of $^{\bullet}OH$ radicals [123–126]. It seems that the presence or absence of Cr(III)–DNA complexes among the products of the Cr(III) + $H_2O_2$ + DNA reactions may be used as a strong argument for either Cr(V/IV) complexes or oxygen-based radicals, respectively, as the main oxidizing species in these systems (to our knowledge, no attempts to detect such complexes have been made as yet).

An important point illustrated by Scheme 8 is that the formation of Cr(III)–DNA adducts (such as **15** or **22**) is likely to have more serious biological consequences than the formation of DNA radicals, such as **16**, as the latter are easily repaired by organic compounds that are abundant in biological media [1, 3, 137]. Transformations of Cr(V)–DNA or Cr(IV)–DNA complexes (**12** or **19**) to Cr(III)–DNA complexes (**15** or **22**) are likely to cause the binding of an additional ligand (**L′** in Scheme 8) to the Cr center (since most of the known Cr(V) or Cr(IV) complexes are five-coordinate and all the Cr(III) complexes are six-coordinate in aqueous solutions) [63]. In cellular systems, this additional ligand is likely to be a protein chain or a second DNA strand, which would cause the formation of highly genotoxic DNA–Cr(III)–protein or DNA–Cr(III)–DNA cross-links [2, 3]. An alternative pathway for the formation of Cr(III)–DNA complexes, illustrated in Scheme 8, involves DNA binding to reactive positively charged Cr(III) complexes, which are formed during the disproportionation of Cr(IV) or Cr(V) complexes (mechanisms of such binding reactions are similar to that shown in Scheme 7a) [115]. The relative importance of this pathway versus the direct reactions of Cr(V/IV) complexes with DNA (shown in the main part of Scheme 8) is currently under dispute [1, 3, 67]; detailed kinetic studies will be required to distinguish between these possibilities. Nevertheless, all the reaction pathways in Scheme 8 clearly show the importance of oxidative activation of Cr(III) (or reductive activation of Cr(VI), Section ‘Introduction’) [1, 3], which leads to reactive Cr(V/IV) intermediates, as a pre-requisite

for the formation of genotoxic Cr(III)–DNA lesions. At the same time, Scheme 7b shows that direct reactions with DNA are unlikely for biologically relevant Cr(III) complexes.

There have been far less in vitro studies on the reactions of the Cr(III) + oxidant systems with proteins or other biomolecules, compared with the studies on similar reactions with DNA. Some Cr(III) complexes, such as $[Cr(salen)(OH_2)_2]^+$ or $[Cr(edta)(OH_2)]^-$ (edta = *N,N,N′N′*-ethanediaminetetraacetate(4-)), induce oxidative degradation of proteins or glycoproteins (bovine serum albumin or human orosomucoid) in the presence of $H_2O_2$, while other complexes, such as $[Cr(en)_3]^{3+}$ (en = 1,2-ethanediamine), did not cause such an effect [142, 143]. Notably, the former two Cr(III) complexes, but not the latter one, can be oxidized to relatively stable Cr(V) species under mild conditions [31]. Recently, Cr(III) complexes with Schiff base ligands (analogs of salen) were shown to induce photo-oxidation of proteins by $O_2$ (in the presence of $N_3^-$), and the formation of Cr(V) complexes during these reactions was detected by EPR spectroscopy [144].

Although mechanisms of protein interactions with the Cr(III) + oxidant systems remain unknown, thiol groups of Cys residues are expected to be the primary targets for the oxidative damage [31]. Reactions of Cr(VI/V/IV) complexes (the likely reactive species formed during the oxidation of Cr(III), Scheme 4) with either free- or Zn-bound biological thiols have been extensively studied using low-molecular-weight model systems [145–148]. The possible influence of these reactions on the regulatory functions of Cys-containing proteins is discussed in Section 'Interference with cell signaling'.

**Interference with cell signaling**

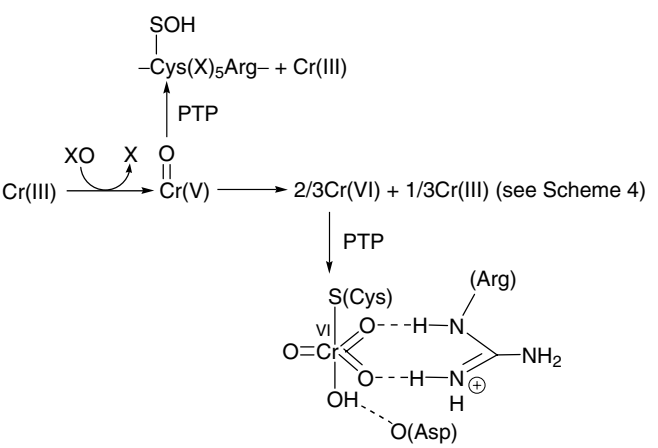
Phosphorylation and dephosphorylation of protein tyrosine residues, catalyzed by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), respectively, are crucial events in many cell signaling pathways, including insulin signaling (for a review, see Refs [149–151]). Therefore, the ability of Cr(III) compounds to affect tyrosine phosphorylation is considered as a likely reason for their anti-diabetic activity [152]. Previously, attention was focused on the suggested ability of some polynuclear Cr(III) complexes, including chromodulin and $[Cr_3O(OCOEt)_6(OH_2)_3]^+$, to enhance the PTK activity of the isolated $\beta$-subunits of human insulin receptors [61, 153]. However, the influence of Cr(III) complexes on PTK assays (based on the use of a phosphotyrosine-specific antibody) [61, 153] may have been caused by their ability to enhance the antibody binding to the test plates [56] (based on the well-known ability of Cr(III) complexes to cross-link proteins) [63, 65]. Indeed, positive results of the PTK assays were also observed in the absence of the enzyme but in the presence of Cr(III) compounds [56, 154].

An alternative pathway for the enhancement of PTK activity of insulin receptors is the inactivation of related PTPs, which is believed to be the basis of anti-diabetic activity of vanadium (V(IV) and V(V)) compounds [155, 156]. The structures and chemical properties of V(IV) and V(V) compounds are similar to those of Cr(V) and Cr(VI) compounds, respectively (since these oxidation states of V and Cr are isoelectronic), except that the Cr(V) and Cr(VI) compounds are stronger oxidants than their V(IV) and V(V) analogs [63, 157]. Active centers of all the known PTPs contain a highly conserved $Cys(X)_5Arg$ motif (where X is any amino acid) [158, 159], and most of the known PTP inhibitors (including V compounds) act by binding to, or oxidation of, the crucial Cys residue [156, 160]. Possible pathways for the reactions of Cr(V) and Cr(VI) species

(formed in the oxidation of Cr(III) complexes, Section 'Oxidation of Cr(III) complexes under physiological conditions') with PTPs are summarized in Scheme 9 [31].

The well-known reversible inhibition of PTPs by vanadate ($[V^{V}O_4]^{3-}$) is based on the formation of a stable five-coordinate V(V) thiolato complex ($[V^{V}O_3(SR)(OH)]^{3-}$, where RSH is the thiol) with the Cys residue in the active center of the enzyme, which acts as a mimic of a transition state of phosphate [156, 161]. Recently, the ability of Cr(VI) to form five-coordinate thiolato complexes in aqueous solutions ($[Cr^{VI}O_3(SR)(OH_2)]^{-}$) has been demonstrated (previously, all the Cr(VI) oxo complexes were thought to be four-coordinate) [148]. This finding implies the ability of Cr(VI) ($[CrO_4]^{2-}$) to inhibit PTPs in a similar way to V(V), which was confirmed experimentally [5] using a well-characterized microbial PTP (from *Yersinia enterocolitica*) [162, 163]. The proposed structure of a Cr(VI)-PTP complex [5], based on the X-ray crystal structure of a V(V)-PTP complex [161] and the structures of Cr(VI) thiolato complexes in aqueous solutions (determined by X-ray absorption spectroscopy) [148], is shown in Scheme 9. In addition to PTP inhibition, Cr(VI) has been recently shown to cause direct activation of some Src family kinases [164], although the chemical mechanisms of such activation are not yet clear.

A model Cr(V) complex, $[Cr^{V}O(ehba)_2]^{-}$ (ehba = 2-ethyl-2-hydroxycarboxylato (2−)), was about an order of magnitude more efficient as a PTP inhibitor in vitro than $[CrO_4]^{2-}$ (based on Cr concentrations) [5]. The mechanism of PTP inhibition by Cr(V) species is likely to be based on the direct oxidation of Cys residues in the active centers of the enzymes (e.g., with the formation of sulfenic acid derivatives, Cys(SOH) in Scheme 9) [5]. Similar PTP inhibition mechanisms have been proposed for $H_2O_2$[160] and V(V) peroxo complexes [156] (which are more potent than vanadate as PTP inhibitors and anti-diabetic agents) [155].




Scheme 9. Possible mechanisms for inactivation of the active centers (–$Cys(X)_5Arg$–, where X is any amino acid) of protein tyrosine phosphatases (PTP) by Cr(V) and Cr(VI) species, formed during the oxidation of Cr(III) complexes (based on the data from Refs [5] and [31]).

There has been a recent explosion of interest in the role of $H_2O_2$ and other ROS in cellular signal transduction [25, 160, 165]. The role of $H_2O_2$ as a physiological messenger molecule via reversible oxidation of Cys residues in PTPs and other regulatory enzymes has been proposed [160]. However, only a few types of such enzymes have Cys residues that are reactive enough to be oxidized efficiently by sub-micromolar concentrations of $H_2O_2$, which are kept to such low levels in most cells by catalase and peroxidases (vide infra) [25]. Increased intracellular concentrations of Cr(III) species, which are known to catalyse oxidations of organic compounds by $H_2O_2$ (vide infra) [92], may lead to increases in the rates of oxidation of Cys residues in the active sites of PTPs, which in turn could cause disruptions in cell signaling pathways. To our knowledge, no experimental studies on the influence of Cr(III) compounds on the rates of reactions of Cys-containing proteins or model thiols with $H_2O_2$ have been performed as yet.

Several studies [61, 166, 167] suggested that PTP activity can be altered by direct reactions with Cr(III) compounds. Opposite effects (inhibition by ~70 % or activation by ~50 %, respectively) of $CrCl_3$ (0.10 mM) were observed in vitro for two recombinant human PTPs involved in insulin signaling (PTPB1 and LAR) [167]. A study published in 2001 [167] did not confirm earlier reports [61, 166] on the activation of membrane PTPs by chromodulin or its structural model ($[Cr_3O(OCOEt)_6(OH_2)_3]^+$). The effects of relatively high (0.10 mM) concentrations of $CrCl_3$ on PTPs [167] are likely to be due to the changes in protein conformations caused by non-specific binding of Cr(III) ions (produced by the hydrolysis of $CrCl_3$, Scheme 7a in Section 'Damage to DNA proteins') [1, 5]. No significant effects of Cr(III) compounds (up to 0.50 mM) on the activity of the *Yersinia enterocolitica* PTP were observed [5]. Thus, it appears that Cr(III) oxidation products, but not Cr(III) itself, are able to specifically react with the active centers of PTPs.

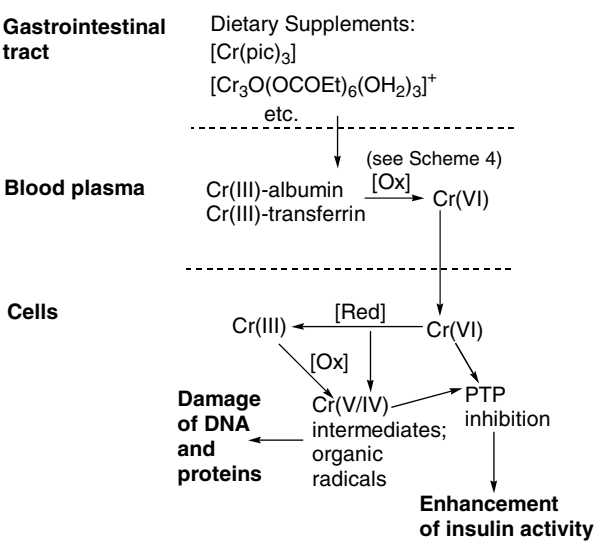
In summary, oxidation of Cr(III) compounds generates Cr(V) and Cr(VI) species that can readily react with Cys residues of PTPs, which leads to increases in the levels of tyrosine phosphorylation and alterations in cell signaling, including an increase in insulin receptor activity [5]. This hypothesis is supported by the observations of increased levels of tyrosine phosphorylation on Cr(VI)-treated mammalian cells [168, 169] and of insulin-mimetic effects of Cr(VI) within animals [170, 171]. On the other hand, disruptions in cell signaling caused by blocking Cys residues of regulatory enzymes are considered among the main reasons for the toxicity of heavy metal compounds, including Cr(VI) [172].

## OXIDATIVE HYPOTHESIS OF Cr(III) BIOLOGICAL ACTIVITY

After five decades of research, the questions remain open on whether Cr(III) is required for normal glucose metabolism, and whether Cr(III)-containing dietary supplements are beneficial in the prevention and control of diabetes (see Sections B and D of this book). The following trends are emerging from the literature surveys published in 2002–2004 [173–176]: (i) Cr(III) supplementation does not cause statistically significant improvements in insulin-related metabolic parameters, and does not assist in weight loss or improvements in body composition in non-diabetic individuals; and (ii) long-term treatments with high doses of Cr(III) (e.g., $\geq$ 200 μg Cr/day as $[Cr(pic)_3]$ for $\geq$ 2 months)

improve glucose tolerance in many (but not all) patients with type II diabetes. Essentiality of Cr(III) for humans was proposed on the basis of significant improvements in glucose tolerance in five patients on total parenteral nutrition (TPN) after the addition of $CrCl_3$ (150–200 μg Cr/day for 3–60 days) to the TPN solutions [177], although this evidence has subsequently been disputed [105]. A study published in 2004 [178] showed possible improvements in glucose metabolism following Cr(III) supplementation in two out of five TPN patients, and little or no effect in the other three. These results are consistent with Cr(III) being a therapeutic agent rather than an essential nutrient [105]. Furthermore, the efficacy of Cr(III) compounds seem to vary greatly between individuals, which is dependent on the presence or absence of a diabetic condition, as well as on some as yet undetermined factors [175, 176].

The observations that: (i) Cr(III) compounds can be oxidized to Cr(VI) species (probably via Cr(V) intermediates) under physiologically relevant conditions (Section 'Oxidation of Cr(III) complexes under physiological conditions'); and (ii) Cr(VI) and Cr(V) compounds act as efficient PTP inhibitors in vitro (Section 'Interference with cell signaling') led us to a hypothesis that the anti-diabetic activities of Cr(III) compounds are caused by their in vivo oxidation to Cr(V) and Cr(VI) species [5]. A possible mechanism of action of Cr(III)-containing nutritional supplements (including the widely used $[Cr(pic)_3]$ and the proposed alternative $[Cr_3O(OCOEt)_6(OH_2)_3]^+$) [152, 175] is illustrated in Scheme 10. The orally taken Cr(III) supplements are absorbed in the small intestine, and are at least partially converted to Cr(III) complexes of serum proteins, such as albumin, and transferrin (see Chapter 1 of this book) [179, 180]. In addition, a stable




Scheme 10. Proposed oxidative mechanism of biological activities of Cr(III) complexes. Designations: [Ox] are biological oxidants, such as $H_2O_2$ or peroxides of proteins and lipids (Section 2) and [Red] are biological reductants, such as glutathione, ascorbate, or NAD(P)H.

chelate complex, $[Cr(pic)_3]$, can be metabolized by hepatic enzymes with the release of more reactive Cr(III) species [181]. Both the initial Cr(III) compounds and their metabolism products (including Cr(III)-protein complexes) can be oxidized to Cr(VI) ($[CrO_4]^{2-}$) by $H_2O_2$, $ClO^-$ or enzymatic systems in biological fluids (Section 'Oxidation of Cr(III) complexes under physiological conditions') [5, 56]. This oxidation is most likely to occur mainly extracellularly, since the cellular uptake of Cr(III) compounds is very slow [1], and the concentrations of strong oxidants are less tightly controlled in extracellular fluids compared with the intracellular environment (Section 'Formation and roles of strong oxidants in biological systems') [9]. The resultant $[CrO_4]^{2-}$ species easily cross cellular membranes through anion channels, and are reduced to Cr(III) complexes by intracellular reductants with the formation of highly reactive Cr(V/IV) intermediates and organic radicals (Section 'Introduction') [1]. These intermediates, as well as Cr(VI) itself, can oxidize Cys residues of PTPs and other regulatory enzymes, causing alterations in cell signaling, including an enhancement in insulin signaling due to the increased tyrosine phosphorylation in $\beta$-subunits of insulin receptors (Section 'Interference with cell signaling with'.) [168]. The Cr(V/IV) intermediates (or their more stable complexes with cellular ligands such as carbohydrates) [81] are also likely to be responsible for the oxidative damage of DNA and proteins, which have the potential to lead to carcinogenic disruptions in gene expression (Section 'Damage to DNA and proteins') [1–3, 67]. Similar Cr(V/IV) species can be generated during the re-oxidation of Cr(III) complexes that are accumulated within the cells (Section 'Oxidation of Cr(III) complexes under physiological conditions') [30, 72].

The synchrotron-radiation-induced X-ray emission (SRIXE) technique, in combination with microprobe X-ray absorption near-edge structure (XANES) spectroscopy, is currently used to follow the uptake, distribution, and changes in the oxidation states of metal complexes, including Cr(III) and Cr(VI) compounds, in individual cells and tissue sections [182–187]. One such study [184] detected a selective accumulation of Cr(III) in testicular tissues of male mice treated intraperitoneally with aqueous $CrCl_3$. This finding is probably related to the toxic effects of such treatment in the progeny [109], although the possible roles of oxidative processes in these effects have not yet been determined. Another study published in 2005 [187] showed that a short-term treatment of cultured human lung epithelial cells with Cr(VI) (0.10 mM for 20 min) leads to the confinement of Cr(III) (formed due to the cellular uptake and reduction of Cr(VI)) in small areas of the cytoplasm (not in the nucleus). This unusual feature was attributed to a new cellular protection mechanism against Cr(VI), since these conditions lead t o nearly 100 % clonogenic survival of the cells (while longer treatments with the same dose of Cr(VI) are highly cytotoxic) [187]. It is possible that a similar mechanism applies to the cellular uptake of small amounts of Cr(VI), formed during the extracellular oxidation of moderate concentrations of Cr(III) (Scheme 10). A situation when Cr(VI) is completely reduced to Cr(III) before it reaches the nucleus [187] (e.g., by the Cys groups of membrane proteins) is likely to lead to the beneficial effects related to the enhanced insulin signaling (vide supra). On the other hand, an excessive uptake of Cr(VI), which overwhelms the protective mechanism and leads to Cr(III) accumulation in the nucleus [185, 187], is likely to be responsible for the toxic effects of Cr(VI) or of high doses of Cr(III) compounds (vide supra). Further studies using the SRIXE and XANES techniques may shed more light onto the redox transformations of Cr compounds in cells and tissues.

The oxidative hypothesis described above (Scheme 10) provides the first unified explanation for the seemingly opposite biological effects of Cr(III) and Cr(VI) compounds [1, 31]. In support of this hypothesis, the reported activities of various Cr(III) complexes as insulin activators seem to correlate with their reactivities toward biological oxidants [5]. For instance, the trinuclear Cr(III) propionate ($[Cr_3O(OCOEt)_6(OH_2)_3]^+$), which is relatively stable toward hydrolysis in neutral aqueous solutions (unlike for $CrCl_3$, Scheme 7a) and is easily oxidized to Cr(VI) under mild conditions (unlike for $[Cr(pic)_3]$) [5], shows the highest insulin-enhancing activity in animal models [57–59, 188]. Generally, the higher activity of Cr(III) compounds in diabetic patients compared with non-diabetics [176] may correlate with the prevalence of oxidative stress in diabetes (vide supra) [27], which leads to depletion of antioxidants in blood plasma and facilitates the extracellular oxidation of Cr(III) to Cr(VI) (Scheme 10). Variations in the anti-diabetic activity of Cr(III) complexes between individuals [176, 178] may be explained by variations in the blood antioxidant levels, caused by nutritional status and/or the presence of inflammatory conditions (vide supra) [7, 9]. The possibility that the anti-diabetic activity of Cr(III) is caused by the generation of a potentially carcinogenic species, Cr(VI), enhances the current concern (see Chapter 9 of this book) about the safety of using Cr(III) compounds as nutritional supplements or therapeutical agents, particularly for certain athletes who consume large amounts of the supplements and for people with diabetes.

## ACKNOWLEDGMENT

Financial support for this work was provided by an Australian Research Council Discovery grant and Australian Professorial Fellowship (to P.A.L.). Irma Mulyani is grateful to Chemistry Department, Bandung Institute of Technology, Indonesia, for partial funding of her postgraduate studies at The University of Sydney.

## REFERENCES

[1] Levina, A., Codd, R., Dillon, C. T., and Lay, P. A. (2003) Chromium in biology: toxicology and nutritional aspects. *Prog. Inorg. Chem. 51*, 145–250.
[2] O'Brien, T. J., Ceryak, S., and Patierno, S. R. (2003) Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms. *Mutat. Res. 533*, 3–36.
[3] Zhitkovich, A. (2005) Importance of chromium-DNA adducts in mutagenicity and toxicity of chromium(VI). *Chem. Res. Toxicol. 18*, 3–11.
[4] Petrilli, F. L., and De Flora, S. (1978) Oxidation of inactive trivalent chromium to the mutagenic hexavalent form. *Mutat. Res. 58*, 167–173.
[5] Mulyani, I., Levina, A., and Lay, P. A. (2004) Biomimetic oxidation of chromium(III): Does the antidiabetic activity of chromium(III) involve carcinogenic chromium(VI)? *Angew. Chem. Int. Ed. 43*, 4504–4507.
[6] Green, M. J., and Hill, H. A. O. (1984) Chemistry of dioxygen. *Methods Enzymol. 105*, 3–22.
[7] Halliwell, B., and Gutteridge, J. M. C. (1999) *Free Radicals in Biology and Medicine*, 3rd ed., Oxford University Press, Oxford, UK.

[8] Branchaud, B. P. (1999) Free radicals as a result of dioxygen metabolism in *Metal Ions in Biological Systems* (Sigel, A., and Sigel, H., eds.) pp. 79–102, Marcel Dekker, New York.
[9] Stocker, R., and Keaney, J. F., Jr. (2004) Role of oxidative modifications in atherosclerosis. *Physiol. Rev. 84*, 1381–1478.
[10] Kochevar, I. E., and Redmond, R. W. (2000) Photosensitized production of singlet oxygen. *Methods Enzymol. 319*, 20–28.
[11] Cadet, J., Douki, T., Pouget, J.-P., and Ravanat, J. L. (2000) Singlet oxygen DNA damage products: Formation and measurements. *Methods Enzymol. 319*, 143–153.
[12] Ahmad, N., and Mukhtar, H. (2000) Mechanism of photodynamic therapy-induced cell death. *Methods Enzymol. 319*, 342–358.
[13] Griendling, K. K., Sorescu, D., and Ushio-Fukai, M. (2000) NAD(P)H oxidase: Role in cardiovascular biology and disease. *Circ. Res. 86*, 494–501.
[14] Vorbach, C., Harrison, R., and Capecchi, M. R. (2003) Xanthine oxidoreductase is central to the evolution and function of the innate immune system. *Trends Immunol. 24*, 512–517.
[15] Berry, C. E., and Hare, J. M. (2004) Xanthine oxidoreductase and cardiovascular disease: Molecular mechanisms and pathophysiological implications. *J. Physiol. 555*, 589–606.
[16] Matyus, P., Dajka-Halasz, B., Foeldi, A., Haider, N., Barlocco, D., and Magyar, K. (2001) Semicarbazide-sensitive amine oxidase: Current status and perspectives. *Curr. Med. Chem. 11*, 1285–1298.
[17] Su, Q., and Klinman, J. P. (1999) Life as aerobes: how does nature control the activation of dioxygen? *Biomed. Health Res. 27*, 20–31.
[18] Avigliano, L., and Finazzi-Agro, A. (1997) Biological function and enzyme kinetics of ascorbate oxidase in *Multi-Copper Oxidases* (Messerschmidt, A., ed.) pp. 251–284, World Scientific, Singapore.
[19] Dunford, H. B. (2002) Oxidations of iron(II)/(III) by hydrogen peroxide: from aquo to enzyme. *Coord. Chem. Rev. 233-234*, 311–318.
[20] Alderton, W. K., Cooper, C. E., and Knowles, R. G. (2001) Nitric oxide synthases: structure, function and inhibition. *Biochem. J. 357*, 593–615.
[21] Squadrito, G. L., and Pryor, W. A. (1998) Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Rad. Biol. Med. 25*, 392–403.
[22] Boccini, F., and Herold, S. (2004) Mechanistic studies of the oxidation of oxyhemoglobin by peroxynitrite. *Biochemistry 43*, 16393–16404.
[23] Hawkins, C. L., and Davies, M. J. (2001) Generation and propagation of radical reactions on proteins. *Biochim. Biophys. Acta 1504*, 196–219.
[24] Cadenas, E. (2004) Mitochondrial free radical production and cell signaling. *Mol. Aspects Med. 25*, 17–26.
[25] Stone, J. R. (2004) An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian systems. *Arch. Biochem. Biophys. 422*, 119–124.
[26] Sies, H. (2000) What is oxidative stress? in *Oxidative Stress and Vascular Disease* (Keaney, J. F. J., ed.) pp. 1–8, Kluwer Academic, Boston.
[27] Robertson, P. R. (2004) Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J. Biol. Chem. 279*, 42351–42354.
[28] Wolff, S. P., and Dean, R. T. (1987) Glucose autoxidation and protein modification. The potential role of "autoxidative glycosylation" in diabetes. *Biochem. J. 245*, 243–250.
[29] Steen, E., Terry, B. M., Rivera, E., Cannon, J. L., Neely, T. R., Tavares, R., Xu, X. J., Wands, J. R., and de la Monte, S. M. (2005) Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease – is this type 3 diabetes? *J. Alzheimer's Dis. 7*, 63–80.
[30] Dillon, C. T., Lay, P. A., Bonin, A. M., Cholewa, M., and Legge, G. J. F. (2000) Permeability, cytotoxicity and genotoxicity of Cr(III) complexes and some Cr(V) analogs in V79 Chinese hamster lung cells. *Chem. Res. Toxicol. 13*, 742–748.

[31] Levina, A., and Lay, P. A. (2004) Mechanistic studies of relevance to the biological activities of chromium. *Coord. Chem. Rev. 249*, 281–298.
[32] Haupt, G. W. (1952) Method for the determination of chromium. *Natl. Bur. Stand. Circ. (U.S.A) 48*, 414–423.
[33] Andersen, J. E. T. (1998) Introduction of hydrogen peroxide as an oxidant in flow injection analysis: speciation of Cr(III) and Cr(VI). *Anal. Chim. Acta 361*, 125–131.
[34] Chatlas, J., Impert, O., Katafias, A., Kita, P., Wrzeszcz, G., Eriksen, J., Mønsted, O., and Mills, A. (2004) Kinetics and mechanism of a macrocyclic Cr(III) complex oxidation to chromium(IV) by hexacyanoferrate(III) in strongly alkaline media. *Trans. Met. Chem. 29*, 634–643.
[35] Anis, S. S., and Mansour, M. A. (2001) Kinetics and mechanisms of the oxidation of nitrilotris(methylenephosphonato)chromium(III) by periodate. *Trans. Met. Chem. 26*, 695–699.
[36] Abdel-Khalek, A. A., and Khaled, E. S. H. (1999) Kinetics and mechanism of oxidation of (ethylenediaminediacetato)chromium(III) by N-bromosuccinimide. *Trans. Met. Chem. 24*, 189–192.
[37] Pal, S. C., and Tarafder, M. T. H. (1996) Kinetics and mechanism of oxidation of two new chromium(III) complexes by hydrogen peroxide. *Indian J. Chem. A 35*, 960–963.
[38] Rajan, R., Nair, B. U., and Ramasami, T. (2000) Hypochlorite oxidation of a chromium(III) Schiff base complex. *Inorg. React. Mech. (Amsterdam) 1*, 247–255.
[39] Funahashi, S., Uchida, F., and Tanaka, M. (1978) Reactions of hydrogen peroxide with metal complexes. 3. Thermodynamic and kinetic studies on the formation, dissociation, and decomposition of peroxochromium(VI) complexes in acid media. *Inorg. Chem. 17*, 2784–2789.
[40] Beck, M. T., Nagy, I. P., and Székely, G. (1991) Oscillatory kinetics and mechanism of the chromate catalyzed decomposition of hydrogen peroxide. *Int. J. Chem. Kinet. 23*, 881–896.
[41] Perez-Benito, J. F., and Arias, C. (1997) A kinetic study of the chromium(VI)-hydrogen peroxide reaction. Role of the diperoxochromate(VI) intermediates. *J. Phys. Chem. A 101*, 4726–4733.
[42] Zhang, L., and Lay, P. A. (1998) EPR spectroscopic studies on the formation of chromium(V) peroxo complexes in the reaction of chromium(VI) with hydrogen peroxide. *Inorg. Chem. 37*, 1729–1733.
[43] Katz, S. A., and Salem, H. (1994) *The Biological and Environmental Chemistry of Chromium*, VCH, New York.
[44] Zayed, A. M., and Terry, N. (2003) Chromium in the environment: factors affecting biological remediation. *Plant Soil 249*, 139–156.
[45] Comber, S., and Gardner, M. (2003) Chromium redox speciation in natural waters. *J. Environ. Monit. 5*, 410–413.
[46] Sander, S., Koschinsky, A., and Halbach, P. (2003) Redox speciation of chromium in the oceanic water column of the Lesser Antilles and offshore Otago Peninsula, New Zealand. *Mar. Freshwater Res. 54*, 745–754.
[47] Ball, J. W., and Izbicki, J. A. (2004) Occurence of hexavalent chromium in ground water in the western Mojave Desert, California. *Appl. Geochem. 19*, 1123–1135.
[48] Banerjee, D., and Nesbitt, H. W. (1999) Oxidation of aqueous Cr(III) at birnessite surfaces: Constraints on reaction mechanism. *Geochim. Cosmochim. Acta 63*, 1671–1687.
[49] Weaver, R. M., and Hochella, M. F. J. (2003) The reactivity of seven Mn-oxides with $Cr^{3+}$aq: A comparative analysis of a complex, environmentally important redox reaction. *Am. Mineral. 88*, 2016–2027.
[50] Pettine, M., and Millero, F. J. (1990) Chromium speciation in seawater: The probable role of hydrogen peroxide. *Limnol. Oceanogr. 35*, 730–736.

[51] Rock, M. L., James, B. R., and Helz, G. R. (2001) Hydrogen peroxide effects on chromium oxidation state and solubility in four diverse, chromium-enriched soils. *Environ. Sci. Technol. 35*, 4054–4059.

[52] Lin, C. J. (2002) The chemical transformation of chromium in natural waters – A model study. *Water, Air Soil Pollut. 139*, 137–158.

[53] Fendorf, S., Wielinga, B. W., and Hansel, C. M. (2000) Chromium transformations in natural environments: The role of biological and abiological processes in chromium(VI) reduction. *Int. Geol. Rev. 42*, 691–701.

[54] Sethunathan, N., Megharaj, M., Smith, L., Kamaludeen, S. P. B., Avudainayagam, S., and Naidu, R. (2005) Microbial role in the failure of natural attenuation of chromium(VI) in long-term tannery waste contaminated soil. *Agricult. Ecosys. Environ. 105*, 657–661.

[55] Knott, H. M., Baoutina, A., Davies, M. J., and Dean, R. T. (2002) Comparative time-courses of copper-ion-mediated protein and lipid oxidation in low-density lipoprotein. *Arch. Biochem. Biophys. 400*, 223–232.

[56] Mulyani, I. (2006) In vitro biological activities and chemistry of Cr(III) dietary supplements, PhD Thesis, The University of Sydney.

[57] Sun, Y. J., Mallya, K., Ramirez, J., and Vincent, J. B. (1999) The biomimetic $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ decreases plasma cholesterol and triglycerides in rats: Towards chromium-containing therapeutics. *J. Biol. Inorg. Chem. 4*, 838–845.

[58] Vincent, J. B., and Davis, C. M. (2001) Use of triaqua-$\mu^3$-oxohexakis-$\mu$-propionatotrichromium(1+), $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$, as a nutritional supplement or in treatment of medical conditions. Patent US 6197816.

[59] Sun, Y. J., Clodfelder, B. J., Shute, A. A., Irvin, T., and Vincent, J. B. (2002) The biomimetic $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ decreases plasma insulin, cholesterol, and triglycerides in healthy and type II diabetic rats but not type I diabetic rats. *J. Biol. Inorg. Chem. 7*, 852–862.

[60] Pickering, A., Chang, C., and Vincent, J. B. (2004) Chromium-containing biomimetic cation triaqua-$\mu^3$-oxo-$\mu$-hexapropionatotrichromium(III) inhibits colorectal tumor formation in rats. *J. Inorg. Biochem. 98*, 1303–1306.

[61] Davis, C. M., Royer, A. C., and Vincent, J. B. (1997) Synthetic multinuclear chromium assembly activates insulin receptor kinase activity – Functional model for low-molecular-weight chromium-binding substance. *Inorg. Chem. 36*, 5316–5320.

[62] Jacquamet, L., Sun, Y., Hatfield, J., Gu, W., Cramer, S. P., Crowder, M. W., Lorigan, G. A., Vincent, J. B., and Latour, J.-M. (2003) Characterization of chromodulin by X-ray absorption and electron paramagnetic resonance spectroscopies and magnetic susceptibility measurements. *J. Am. Chem. Soc. 125*, 774–780.

[63] Lay, P. A., and Levina, A. (2004) Chromium in *Comprehensive Coordination Chemistry II: From Biology to Biotechnology* (McCleverty, J. A., and Meyer, T. J., eds.), Vol. 4, pp. 313–413, Elsevier Science, Amsterdam, The Netherlands.

[64] Chaudhuri, D., Chakraborti, R., and Bhattacharya, P. (2004) How much Cr(VI) is formed during extraction of leather with phosphate buffer? *J. Am. Leather Chem. Assoc. 99*, 119–128.

[65] Brown, E. M., Dudley, R. L., and Elsetinow, A. R. (1997) A conformational study of collagen as affected by tanning procedures. *J. Am. Leather Chem. Assoc. 92*, 225–233.

[66] Long, A. J., Cory, N. J., and Wood, C. B. (2000) Potential chemical mechanisms causing false positive results in hexavalent chromium determination. *J. Soc. Leather. Technol. Chem. 84*, 74–78.

[67] Sugden, K. D., and Stearns, D. M. (2000) The role of chromium(V) in the mechanism of chromate-induced oxidative DNA damage and cancer. *J. Environ. Pathol., Toxicol. Oncol. 19*, 215–230.

[68] Srinivasan, K., and Kochi, J. K. (1985) Synthesis and molecular structure of oxochromium(V) cations. Coordination with donor ligands. *Inorg. Chem. 24*, 4671–4679.

[69] Weeks, C. L., Levina, A., Dillon, C. T., Turner, P., Fenton, R. R., and Lay, P. A. (2004) Synthesis and characterization of a chromium(V) *cis*-dioxobis(1,10-phenanthroline) complex and crystal and molecular structures of its chromium(III) precursor. *Inorg. Chem. 43*, 7844–7856.

[70] Headlam, H. A. (1998) *The role of Cr(III) and Cr(V) peptide and amino acid complexes in Cr-induced carcinogenesis.* PhD Thesis, The University of Sydney.

[71] Barnard, P., Levina, A., and Lay, P. A. (2005) Chromium(V) peptide complexes: Synthesis and spectroscopic characterization. *Inorg. Chem. 44*, 1044–1053.

[72] Dillon, C. T., Lay, P. A., Bonin, A. M., Dixon, N. E., and Sulfab, Y. (2000) DNA interactions and bacterial mutagenicity of some chromium(III) imine complexes and their chromium(V) analogues. Evidence for chromium(V) intermediates in the genotoxicity of chromium(III). *Aust. J. Chem. 53*, 411–424.

[73] Bakac, A., and Wang, W.-D. (1996) Biomimetic chemistry of chromium. Intramolecular conversion of hydroperoxochromium(III) to oxochromium(V). *J. Am. Chem. Soc. 118*, 10325–10326.

[74] Pestovsky, O., and Bakac, A. (2005) Oxygen activation by a macrocyclic chromium complex. Mechanism of hydroperoxo-chromium(III) to oxo-chromium(V) transformation. *Dalton Trans.*, 556–560.

[75] Sugden, K. D., Campo, C. K., and Martin, B. D. (2001) Direct oxidation of guanine and 7,8-dihydro-8-oxoguanine in DNA by a high-valent chromium complex: A possible mechanism for chromate genotoxicity. *Chem. Res. Toxicol. 14*, 1315–1322.

[76] Bakac, A., and Wang, W.-D. (2000) Generation of a macrocyclic Cr(V) complex and its reactivity toward organic and inorganic reductants and DNA. *Inorg. Chim. Acta 297*, 27–35.

[77] Sugden, K. D., Burris, R. B., and Rogers, S. J. (1990) An oxygen dependence in chromium mutagenesis. *Mutat. Res. 244*, 239–244.

[78] Ortiz de Montellano, P. R. E. (1995) *Cytochrome P450: Structure, Mechanism, and Biochemistry*, 2nd ed., Plenum Press, New York.

[79] Enemark, J. H., Cooney, J. J. A., Wang, J. J., and Holm, R. H. (2004) Synthetic analogues and reaction systems relevant to the molybdenum and tungsten oxotransferases. *Chem. Rev. 104*, 1175–1200.

[80] Krumpolc, M., and Roček, J. (1985) Cr(V) Oxidations of organic compounds. *Inorg. Chem. 24*, 617–621.

[81] Codd, R., Irwin, J. A., and Lay, P. A. (2003) Sialoglycoprotein and carbohydrate complexes in chromium toxicity. *Curr. Opin. Chem. Biol. 7*, 213–219.

[82] Sugden, K. D., Geer, R. D., and Rogers, S. J. (1992) Oxygen radical-mediated DNA damage by redox-active Cr(III) complexes. *Biochemistry 31*, 11626–11631.

[83] Speetjens, J. K., Collins, R. A., Vincent, J. B., and Woski, S. A. (1999) The nutritional supplement chromium(III) tris(picolinate) cleaves DNA. *Chem. Res. Toxicol. 12*, 483–487.

[84] Chaudhary, S., Pinkston, J., Rabile, M. M., and Van Horn, J. D. (2005) Unusual reactivity in a commercial chromium supplement compared to baseline DNA cleavage with synthetic chromium complexes. *J. Inorg. Biochem. 99*, 787–794.

[85] Cawich, C. M., Ibrahim, A., Link, K. L., Bumgartner, A., Patro, M. D., Mahapatro, S. N., Lay, P. A., Levina, A., Eaton, S. S., and Eaton, G. R. (2003) Synthesis of a pyridinium bis(citrato(2-))oxochromate(V) complex and its ligand-exchange reactions. *Inorg. Chem. 42*, 6458–6468.

[86] Codd, R., Lay, P. A., and Levina, A. (1997) Stability and ligand exchange reactions of chromium(IV) carboxylato complexes in aqueous solutions. *Inorg. Chem. 36*, 5440–5448.

[87] Shi, X., Dalal, N. S., and Kasprzak, K. S. (1993) Generation of free radicals from hydrogen peroxide and lipid hydroperoxides in the presence of chromium(III). *Arch. Biochem. Biophys. 302*, 294–299.

[88] Shi, X., Leonard, S. S., Liu, K. J., Zang, L., Gannett, P. M., Rojanasakul, Y., Castranova, V., and Vallyathan, V. (1998) Cr(III)-mediated hydroxyl radical generation via Haber-Weiss cycle. *J. Inorg. Biochem. 69*, 263–268.

[89] Sugden, K. D., and Wetterhahn, K. E. (1996) Reaction of chromium(V) with the EPR spin traps 5,5-dimethylpyrroline *N*-oxide and phenyl-*N*-*tert*-butylnitrone resulting in direct oxidation. *Inorg. Chem. 35*, 651–657.

[90] Liochev, S. I. (1999) The mechanism of "Fenton-like" reactions and their importance for biological systems. A biologist's view in *Metal Ions in Biological Systems* (Sigel, A., and Sigel, H., eds.) pp. 1–40, Marcel Dekker, New York.

[91] Burkitt, M. J. (2003) Chemical, biological and medical controversies surrounding the Fenton reaction. *Prog. React. Kinet. Mech. 28*, 75–103.

[92] Meseguer-Lloret, S., Campíns-Falcó, P., Tortajada-Genaro, L. A., and Blasco-Gómes, F. (2003) A guide to avoid method bias of chromium(III, VI) chemiluminescence determination by luminol – hydrogen peroxide reaction – application to water samples. *Int. J. Environ. Anal. Chem. 83*, 405–416.

[93] Jain, S. K., and Kannan, K. (2001) Chromium chloride inhibits oxidative stress and TNF-$\alpha$ secretion caused by exposure to high glucose in cultured U937 monocytes. *Biochem. Biophys. Res. Commun. 289*, 687–691.

[94] Vinson, J. A., Mandarano, M. A., Shuta, D. L., Bagchi, M., and Bagchi, D. (2002) Beneficial effects of a novel IH636 grape seed proanthocyanidin extract and a niacin-bound chromium in a hamster atherosclerosis model. *Molec. Cell. Biochem. 240*, 99–103.

[95] Anderson, R. A., Roussel, A.-M., Zouari, N., Mahjoub, S., Matheau, J.-M., and Kerkeni, A. (2001) Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. *J. Am. Coll. Nutr. 20*, 212–218.

[96] Cheng, H.-H., Lai, M.-H., Hou, W.-C., and Huang, C.-L. (2004) Antioxidant effects of chromium supplementation with type 2 diabetes mellitus and euglycemic subjects. *J. Agric. Food Chem. 52*, 1385–1389.

[97] Bagchi, D., Stohs, S. J., Downs, B. W., Bagchi, M., and Preuss, H. G. (2002) Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology 180*, 5–22.

[98] Hepburn, D. D. D., Burney, J. M., Woski, S. A., and Vincent, J. B. (2003) The nutritional supplement chromium picolinate generates oxidative DNA damage and peroxidized lipids in vivo. *Polyhedron 22*, 455–463.

[99] Petit, A., Mwale, F., Tkaczyk, C., Antoniou, J., Zukor, D. J., and Huk, O. L. (2005) Induction of protein oxidation by cobalt and chromium ions in human U937 macrophages. *Biomaterials 26*, 4416–4422.

[100] Tezuka, M., Momiyama, K., Edano, T., and Okada, S. (1991) Protective effect of chromium(III) on acute lethal toxicity of carbon tetrachloride in rats and mice. *J. Inorg. Biochem. 42*, 1–8.

[101] Jain, S. K., Rogier, K., Prouty, L., and Jain, S. K. (2004) Protective effects of 17β-estradiol and trivalent chromium on interleukin-6 secretion, oxidative stress, and adhesion of monocytes: relevance to heart disease in postmenopausal women. *Free Rad. Biol. Med. 37*, 1730–1735.

[102] De Mattia, G., Bravi, M. C., Laurenti, O., De Luca, O., Palmeri, A., Sabatucci, A., Mendico, G., and Ghiselli, A. (2004) Impairment of cell and plasma redox state in subject professionally exposed to chromium. *Am. J. Ind. Med. 46*, 120–125.

[103] Medeiros, M. G., Rodrigues, A. S., Batoreu, M. C., Laires, A., Zhitkovich, A., and Rueff, J. (2003) Biomarkers of chromium exposure and cytogenetic damage in leather tanning and welding industry workers. *NATO Science Ser., Ser. I 351*, 132–141.

[104] Kingry, K. F., Royer, A. C., and Vincent, J. B. (1998) Nuclear magnetic resonance studies of chromium(III) pyridinecarboxylate complexes. *J. Inorg. Biochem. 72*, 79–88.

[105] Stearns, D. M. (2000) Is chromium a trace essential metal? *BioFactors 11*, 149–162.
[106] Speetjens, J. K., Parand, A., Crowder, M. W., Vincent, J. B., and Woski, S. A. (1999) Low-molecular-weight chromium-binding substance and biomimetic $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$ do not cleave DNA under physiologically-relevant conditions. *Polyhedron 18*, 2617–2624.
[107] Anderson, R. A., Polansky, M. M., and Bryden, N. A. (2004) Stability and absorption of chromium and absorption of chromium histidinate complexes by humans. *Biol. Trace Elem. Res. 101*, 211–218.
[108] Yang, X., Palanichamy, K., Ontko, A. C., Rao, M. N. A., Fang, C. X., Ren, J., and Sreejayan, N. (2005) A newly synthetic chromium complex – chromium(phenylalanine)$_3$ improves insulin responsiveness and reduces whole body glucose tolerance. *FEBS Lett. 579*, 1458–1464.
[109] Yu, W., Sipowicz, M. A., Haines, D. C., Birely, L., Diwan, B. A., Riggs, C. W., Kasprzak, K. S., and Anderson, L. M. (1999) Preconception urethane or chromium(III) treatment of male mice: Multiple neoplastic and non-neoplastic changes in offspring. *Toxicol. Appl. Pharmacol. 158*, 161–176.
[110] Beyersmann, D., and Köster, A. (1987) On the role of trivalent Cr in Cr-genotoxicity. *Toxicol. Environ. Chem. 14*, 11–22.
[111] Wolf, T., Kasemann, R., and Ottenwalder, H. (1989) Molecular interaction of different chromium species with nucleotides and nucleic acids. *Carcinogenesis 10*, 655–659.
[112] Gulanowski, B., Swiatek, J., and Kozlowski, H. (1992) Impact of chromium ions on nucleoside triphosphates and nucleic acids. *J. Inorg. Biochem. 48*, 289–298.
[113] Arakawa, H., Ahmad, R., Naoui, M., and Tajmir-Riahi, H. A. (2000) A comparative study of calf thymus DNA binding to Cr(III) and Cr(VI) ions – Evidence for the guanine *N*-7-chromium-phosphate chelate formation. *J. Biol. Chem. 275*, 10150–10153.
[114] Blankert, S. A., Coryell, V. H., Picard, B. T., Wolf, K. K., Lomas, R. E., and Stearns, D. M. (2003) Characterization of nonmutagenic Cr(III)-DNA interactions. *Chem. Res. Toxicol. 16*, 847–854.
[115] Levina, A., Lay, P. A., and Dixon, N. E. (2001) Disproportionation of a model chromium(V) complex causes extensive chromium(III)-DNA binding in vitro. *Chem. Res. Toxicol. 14*, 946–950.
[116] Köster, A., and Beyersmann, D. (1985) Chromium binding by calf thymus nuclei and effects on chromatin. *Toxicol. Environ. Chem. 10*, 307–313.
[117] Stearns, D. M., Belbruno, J. J., and Wetterhahn, K. E. (1995) A prediction of chromium(III) accumulation in humans from chromium dietary supplements. *FASEB J. 9*, 1650–1657.
[118] Kane-Maguire, N. A. P., and Wheeler, J. F. (2001) Photoredox behavior and chiral discrimination of DNA bound M(diimine)$_3^{n+}$ complexes ($M = Ru^{2+}$, $Cr^{3+}$). *Coord. Chem. Rev. 211*, 145–162.
[119] Vaidyanathan, V. G., and Nair, B. U. (2004) Nucleobase oxidation of DNA by (terpyridyl)chromium(III) derivatives. *Eur. J. Inorg. Chem.*, 1840–1846.
[120] Wollfe, S. (1992) *Chromatin: Structure and Function*, Academic Press, London.
[121] Kortenkamp, A., Curran, B., and O'Brien, P. (1992) Defining conditions for the efficient in vitro cross-linking of proteins to DNA by Cr(III) compounds. *Carcinogenesis 13*, 307–308.
[122] Hneihen, A. S., Standeven, A. M., and Wetterhahn, K. E. (1993) Differential binding of chromium(VI) and chromium(III) complexes to salmon sperm nuclei and nuclear DNA and isolated calf thymus DNA. *Carcinogenesis 14*, 1795–1803.
[123] Tsou, T.-C., and Yang, J.-L. (1996) Formation of reactive oxygen species and DNA strand breakage during interaction of chromium(III) and hydrogen peroxide in vitro: Evidence for a chromium(III)-mediated Fenton-like reaction. *Chem.-Biol. Interact. 102*, 133–153.

[124] Tsou, T.-C., Chen, C.-L., Liu, T.-Y., and Yang, J.-L. (1996) Induction of 8-hydroxydeoxyguanosine in DNA by chromium(III) plus hydrogen peroxide and its prevention by scavengers. *Carcinogenesis 17*, 103–108.

[125] Lloyd, D. R., Carmichael, P. L., and Phillips, D. H. (1998) Comparison of the formation of 8-Hydroxy-2′-deoxyguanosine and single- and double-strand breaks in DNA mediated by Fenton reactions. *Chem. Res. Toxicol. 11*, 420–427.

[126] Qi, W. B., Reiter, R. J., Tan, D. X., Garcia, J. J., Manchester, L. C., Karbownik, M., and Calvo, J. R. (2000) Chromium(III)-induced 8-hydroxydeoxyguanosine in DNA and its reduction by antioxidants: Comparative effects of melatonin, ascorbate, and vitamin E. *Environ. Health Persp. 108*, 399–402.

[127] Qi, W. B., Reiter, R. J., Tan, D. X., Manchester, L. C., Siu, A. W., and Garcia, J. J. (2000) Increased levels of oxidatively damaged DNA induced by chromium(III) and $H_2O_2$: Protection by melatonin and related molecules. *J. Pineal Res. 29*, 54–61.

[128] Burkhardt, S., Reiter, R. J., Tan, D. X., Hardeland, R., Cabrera, J., and Karbownik, M. (2001) DNA oxidatively damaged by chromium(III) and $H_2O_2$ is protected by the antioxidants melatonin, *N*-1-acetyl-*N*-2-formyl-5-methoxykynuramine, resveratrol and uric acid. *Int. J. Biochem. Cell Biol. 33*, 775–783.

[129] López-Burillo, S., Tan, D.-X., Mayo, J. C., Sainz, R. M., Manchester, L. C., and Reiter, R. J. (2003) Melatonin, xanthurenic acid, resveratrol, EGCG, vitamin C and α-lipoic acid differentially reduce oxidative DNA damage induced by Fenton reagents: A study of their individual and synergistic actions. *J. Pineal Res. 34*, 269–277.

[130] Parand, A., Royer, A. C., Cantrell, T. L., Weitzel, M., Memon, N., Vincent, J. B., and Crowder, M. W. (1998) DNA nicking by a trinuclear chromium complex. *Inorg. Chim. Acta 268*, 211–219.

[131] Sreedhara, A., Susa, N., and Rao, C. P. (1997) Vanadate and chromate reduction by saccharides and L-ascorbic acid: effect of the isolated V(IV) and Cr(III) products on DNA nicking, lipid peroxidation, cytotoxicity and on enzymatic and non-enzymatic antioxidants. *Inorg. Chim. Acta 263*, 189–194.

[132] Sugden, K. D., and Wetterhahn, K. E. (1996) Identification of the oxidized products formed upon reaction of chromium(V) with thymidine nucleotides. *J. Am. Chem. Soc. 118*, 10811–10818.

[133] Sugden, K. D., and Wetterhahn, K. E. (1997) Direct and hydrogen peroxide-induced chromium(V) Oxidation of Deoxyribose in Single-Stranded and Double-Stranded Calf Thymus DNA. *Chem. Res. Toxicol. 10*, 1397–1406.

[134] Bose, R. N., Fonkeng, B. S., Moghaddas, S., and Stroup, D. (1998) Mechanisms of DNA damage by chromium(V) carcinogens. *Nucleic Acids Res. 26*, 1588–1596.

[135] Bose, R. N., Moghaddas, S., Mazzer, P. A., Dudones, L. P., Joudah, L., and Stroup, D. (1999) Oxidative damage of DNA by chromium(V) complexes: Relative importance of base versus sugar oxidation. *Nucleic Acids Res. 27*, 2219–2226.

[136] Sugden, K. D. (1999) Formation of modified cleavage termini from the reaction of chromium(V) with DNA. *J. Inorg. Biochem. 77*, 177–183.

[137] Levina, A., Barr-David, R., Codd, R., Lay, P. A., Dixon, N. E., Hammershøi, A., and Hendry, P. (1999) In vitro plasmid DNA cleavage by chromium(V) and -(IV) 2-hydroxycarboxylato complexes. *Chem. Res. Toxicol. 12*, 371–381.

[138] Levina, A., Lay, P. A., and Dixon, N. E. (2000) Disproportionation and nuclease activity of bis[2-ethyl-2-hydroxybutanoato(2−)]oxochromate(V) in neutral aqueous solutions. *Inorg. Chem. 39*, 385–395.

[139] Sugden, K. D., and Wetterhahn, K. E. (1996) EPR evidence for chromium(V) binding to phosphate and pyrophosphate: Implications for chromium(V)-DNA interactions. *Inorg. Chem. 35*, 3727–3728.

[140] Zhang, L., and Lay, P. A. (1996) EPR spectroscopic studies of the reactions of Cr(VI) with L-ascorbic acid, L-dehydroascorbic acid and 5,6-*O*-isopropylidene-L-ascorbic acid in water. Implications for chromium(VI) genotoxicity. *J. Am. Chem. Soc. 118*, 12624–12637.
[141] Pattison, D. I., Davies, M. J., Levina, A., Dixon, N. E., and Lay, P. A. (2001) Chromium(VI) reduction by catechol(amine)s results in DNA cleavage in vitro: relevance to chromium genotoxicity. *Chem. Res. Toxicol. 14*, 500–510.
[142] Shrivastava, H. Y., and Nair, B. U. (2000) Protein degradation by peroxide catalyzed by chromium(III): Role of coordinated ligand. *Biochem. Biophys. Res. Commun. 270*, 749–754.
[143] Shrivastava, H. Y., and Nair, B. U. (2001) Chromium(III)-mediated structural modification of glycoprotein: Impact of the ligand and the oxidants. *Biochem. Biophys. Res. Commun. 285*, 915–920.
[144] Shrivastava, H. Y., and Nair, B. U. (2004) Fluorescence resonance energy transfer from tryptophan to a chromium(III) complex accompanied by non-specific cleavage of albumin: a step forward towards the development of a novel photoprotease. *J. Inorg. Biochem. 98*, 991–994.
[145] Lay, P. A., and Levina, A. (1996) Kinetics and mechanism of chromium(VI) reduction to chromium(III) by L-cysteine in neutral aqueous solutions. *Inorg. Chem. 35*, 7709–7717.
[146] Lay, P. A., and Levina, A. (1998) Activation of molecular oxygen during the reactions of chromium(VI/V/IV) with biological reductants: Implications for chromium-induced genotoxicities. *J. Am. Chem. Soc. 120*, 6704–6714.
[147] Levina, A., Bailey, A. M., Champion, G., and Lay, P. A. (2000) Reactions of chromium(VI/V/IV) with bis(*O*-ethyl-L-cysteinato-*N,S*)zinc(II): A model for the action of carcinogenic chromium on zinc-finger proteins. *J. Am. Chem. Soc. 122*, 6208–6216.
[148] Levina, A., and Lay, P. A. (2004) Solution structures of chromium(VI) complexes with glutathione and model thiols. *Inorg. Chem. 43*, 324–335.
[149] Goffin, V., and Kelly, P. A. (2002) Protein phosphorylation and protein-protein interactions in *Hormone Signaling* (Goffin, V., and Kelly, P. A., eds.) pp. 3–20, Kluwer Academic Publishers, Boston MA.
[150] Zhao, Y., and Zhang, Z.-Y. (2002) Control of signaling by tyrosine phosphatases in *Hormone signaling* (Goffin, V., and Kelly, P. A., eds.) pp. 21–36, Kluwer Academic Publishers, Boston MA.
[151] LeRoith, D., Quon, M. J., and Zick, Y. (2002) Insulin and insulin-like growth factor-1 receptors and signaling pathways: Similarities and differences in *Hormone Signaling* (Goffin, V., and Kelly, P. A., eds.) pp. 81–100, Kluwer Academic Publishers, Boston MA.
[152] Vincent, J. B. (2004) Recent developments in the biochemistry of chromium(III). *Biol. Trace Elem. Res. 99*, 1–16.
[153] Davis, C. M., and Vincent, J. B. (1997) Chromium oligopeptide activates insulin receptor tyrosine kinase activity. *Biochemistry 36*, 4382–4385.
[154] Davis, C. M. (1997) *Low-molecular-weight chromium-binding substance: the link between chromium and diabetes.* PhD Thesis, The University of Alabama.
[155] Thompson, K. H., McNeill, J. H., and Orvig, C. (1999) Vanadium compounds as insulin mimics. *Chem. Rev. 99*, 2561–2571.
[156] Huyer, G., Liu, S., Kelly, J., Moffat, J., Payette, P., Kennedy, B., Tsaprailis, G., Gresser, M. J., and Ramachandran, C. (1997) Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate. *J. Biol. Chem. 272*, 843–851.
[157] Crans, D. C., and Smee, J. J. (2004) Vanadium in *Comprehensive Coordination Chemistry II: From Biology to Biotechnology* (McCleverty, J. A., and Meyer, T. J., eds.) Vol 4, pp. 175–239, Elsevier Science, Amsterdam, The Netherlands.
[158] Zhang, Z.-Y., Wang, Y., Wu, L., Fauman, E. B., Stuckey, J. A., Schubert, H. L., Saper, M. A., and Dixon, J. E. (1994) The $Cys(X)_5Arg$ catalytic motif in phosphoester hydrolysis. *Biochemistry 33*, 15266–15270.

[159] Denu, J. M., and Dixon, J. E. (1998) Protein tyrosine phosphatases: Mechanisms of catalysis and regulation. *Curr. Opin. Chem. Biol. 2*, 633–641.

[160] Denu, J. M., and Tanner, K. G. (1998) Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: Evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry 37*, 5633–5642.

[161] Zhang, M., Zhou, M., Van Etten, R. L., and Stauffacher, C. V. (1997) Crystal structure of bovine low molecular weight phosphotyrosyl phosphatase complexes with the transition state analog vanadate. *Biochemistry 36*, 15–23.

[162] Zhang, Z.-Y., Clemens, J. C., Schubert, H. L., Stuckey, J. A., Fischer, M. W. F., Hume, D. M., Saper, M. A., and Dixon, J. E. (1992) Expression, purification and physicochemical characterization of a recombinant *Yersinia* protein tyrosine phosphatase. *J. Biol. Chem. 267*, 23759–23766.

[163] Stuckey, J. A., Schubert, H. L., Fauman, E. B., Zhang, Z.-Y., Dixon, J. E., and Saper, M. E. (1994) Crystal structure of *Yersinia* protein tyrosine phosphatase at 2.5 Å and the complex with tungstate. *Nature 370*, 571–575.

[164] O'Hara, K. A., Klei, L. R., and Barchowsky, A. (2003) Selective activation of Src family kinases and JNK by low levels of chromium(VI). *Toxicol. Appl. Pharmacol. 190*, 214–223.

[165] Poli, G., Leonarduzzi, G., Biasi, F., and Chiarpotto, E. (2004) Oxidative stress and cell signaling. *Curr. Med. Chem. 2004*, 1163–1182.

[166] Davis, C. M., Sumrall, K. H., and Vincent, J. B. (1996) A biologically active form of chromium may activate a membrane phosphotyrosine phosphatase. *Biochemistry 35*, 12963–12969.

[167] Goldstein, B. J., Zhu, L., Hager, R., Zilbering, A., Sun, Y. J., and Vincent, J. B. (2001) Enhancement of post-receptor insulin signaling by trivalent chromium in hepatoma cells is associated with differential inhibition of specific protein-tyrosine phosphatases. *J. Trace Met. Exp. Med. 14*, 393–404.

[168] Yurkow, E. J., and Kim, G. (1995) Effects of chromium on basal and insulin-induced tyrosine phosphorylation in H4 hepatoma cells – comparison with phorbol-12-myristate-13-acetate and sodium orthovanadate. *Molec. Pharmacol. 47*, 686–695.

[169] Qian, Y., Jiang, B., Flynn, D. C., Leonard, S. S., Wang, S. W., Zhang, Z., Ye, J. P., Chen, F., Wang, L. Y., and Shi, X. L. (2001) Cr(VI) increases tyrosine phosphorylation through reactive oxygen species-mediated reactions. *Molec. Cell. Biochem. 222*, 199–204.

[170] Kim, E., and Na, K. J. (1991) Effect of sodium dichromate on carbohydrate metabolism. *Toxicol. Appl. Pharmacol. 110*, 251–258.

[171] Goto, Y., and Kida, K. (1995) Insulin-like action of chromate on glucose transport in isolated rat adipocytes. *Jap. J. Pharmacol. 67*, 365–368.

[172] Barchowsky, A., and O'Hara, K. A. (2003) Metal-induced cell signaling and gene activation in lung diseases. *Free Rad. Biol. Med. 34*, 1130–1135.

[173] Althuis, M. D., Jordan, N. E., Ludington, E. A., and Wittes, J. T. (2002) Glucose and insulin responses to dietary chromium supplements: A meta-analysis. *Am. J. Clin. Nutr. 76*, 148–155.

[174] Pittler, M. H., Stevinson, C., and Ernst, E. (2003) Chromium picolinate for reducing body weight: Meta-analysis of randomized trials. *Int. J. Obes. 27*, 522–529.

[175] Vincent, J. B. (2004) Recent advances in the nutritional biochemistry of trivalent chromium. *Proc. Nutr. Soc. 63*, 41–47.

[176] Cefalu, W. T., and Hu, F. B. (2004) Role of chromium in human health and in diabetes. *Diabetes Care 27*, 2741–2751.

[177] Jeejeebhoy, K. N. (1999) Chromium and parenteral nutrition. *J. Trace Elem. Exp. Med. 12*, 85–89.

[178] Wongseelashote, O., Daly, M. A., and Frankel, E. H. (2004) High insulin requirement versus high chromium requirement in patients nourished with total parenteral nutrition. *Nutrition 20*, 318–320.

[179] Borguet, F., Cornelis, R., Delanghe, J., Lambert, M.-C., and Lameire, N. (1995) Study of the chromium binding in plasma of patients on continuous ambulatory peritoneal dialysis. *Clin. Chim. Acta 238*, 71–84.

[180] Clodfelder, B. J., Upchurch, R. G., and Vincent, J. B. (2004) A comparison of the insulin-sensitive transport of chromium in healthy and model diabetic rats. *J. Inorg. Biochem. 98*, 522–533.

[181] Kareus, S. A., Kelley, C., Walton, H. S., and Sinclair, P. R. (2001) Release of Cr(III) from Cr(III) picolinate upon metabolic activation. *J. Hazard. Mater. 84*, 163–174.

[182] Dillon, C. T., Lay, P. A., Cholewa, M., Legge, G. J. F., Bonin, A. M., Collins, T. J., Kostka, K. L., and Shea-McCarthy, G. (1997) Microprobe X-ray absorption spectroscopic determination of the oxidation state of intracellular chromium following exposure of V79 Chinese hamster lung cells to genotoxic chromium Complexes. *Chem. Res. Toxicol. 10*, 533–535.

[183] Kitamura, N., and Ektessabi, A. M. (2001) XAFS in a single macrophage cell. *J. Synchrotron Rad. 8*, 981–983.

[184] Ortega, R., Devès, G., Bonnin-Mosbah, M., Salomé, M., Susini, J., Anderson, L. M., and Kasprzak, K. S. (2001) Chromium mapping in male mice reproductive glands exposed to $CrCl_3$ using proton and X-ray synchrotron radiation microbeams. *Nucl. Instrum. Methods Phys. Res., Sect. B 181*, 485–488.

[185] Dillon, C. T., Lay, P. A., Kennedy, B. J., Stampfl, A. P. J., Cai, Z., Ilinski, P., Rodrigues, W., Legnini, D. G., Lai, B., and Maser, J. (2002) Hard X-ray microprobe studies of chromium(VI)-treated V79 Chinese hamster lung cells: Intracellular mapping of the biotransformation product of a chromium carcinogen. *J. Biol. Inorg. Chem. 7*, 640–645.

[186] Ortega, R., Devès, G., Fayard, B., Salomé, M., and Susisni, J. (2003) Combination of synchrotron radiation X-ray microprobe and nuclear microprobe for chromium and chromium oxidation states quantitative mapping in single cells. *Nucl. Instrum. Methods Phys. Res., Sect. B 210*, 325–329.

[187] Harris, H. H., Levina, A., Dillon, C. T., Mulyani, I., Lai, B., Cai, Z., and Lay, P. A. (2005) Time-dependent uptake, distribution and biotransformation of chromium(VI) in individual human lung cells: Application of synchrotron radiation techniques. *J. Biol. Inorg. Chem. 10*, 105–118.

[188] Vincent, J. B., Clodfelder, B. J., and Gullick, B. M. (2004) Oral adminsitration of chromium-containing biomimetic complex increases insulin sensitivity and improves plasma lipids in healthy and diabetic rats in *Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA*. Abstr. INOR-640.

*Chapter 12*

# Overview of chromium(III) toxicology

**Qingdong Ke and Max Costa***

Nelson Institute of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, NY 10987

## INTRODUCTION

Chromium (Cr) is abundant in the crust of the Earth and ubiquitously exists in the environment. It occurs naturally in the soil, the rock, and living organisms [1]. It can also be generated by various modern industries, such as metal finishing, welding, plating, wood preservation, water cooling, leather tanning, and production of textiles, dyes, and pigments [2, 3]. In addition, cigarette smoke and automobile emissions contribute to the non-occupational exposure to Cr [3].

Environmental exposure to Cr is becoming an increasing concern, and certain Cr compounds are recognized as human carcinogens. In the periodic table, Cr belongs to the first transition element in group VIB. There are two major stable oxidation states of Cr, hexavalent Cr(VI) and trivalent Cr(III). At physiological conditions, Cr(VI) exists as a chromate oxyanion $CrO_4^{2-}$, which resembles phosphate and sulfate. Thereby, it can be taken up by cells through non-specific phosphate/sulfate anionic transporters [4, 5]. Once entering cells, Cr(VI) is rapidly reduced to Cr(V) or Cr(IV) and ultimately to Cr(III) by low-molecular-weight thiols such as reduced glutathione (GSH) and cysteine (Cys). Depending upon reductant and substrate concentrations, it is reduced rapidly by two electron reduction to Cr(III) without any intermediates by ascorbic acid [6–8]. The intermediate oxidation states of Cr, Cr(IV) and Cr(V), are extremely unstable, and it is the final oxidation state Cr(III) that exists in biological systems. Chromium(III) contains six coordination sites and is capable of forming complexes with ligands and interacting with cellular macromolecules such as DNA, RNA, lipids, and proteins [9, 10]. Epidemiological studies have shown that occupational exposure to Cr(VI) compounds is associated with an increased risk of lung cancer [11]. It has also been shown, from both animal and cell culture studies, that Cr(VI) compounds are carcinogenic. The International Agency for Research on Cancer (IARC) and the US EPA have classified Cr(VI)-containing compounds as Group 1 and Group A human carcinogens, respectively [12].

* The invited author (and primary author) of this chapter.

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

Although Cr(III) is the ultimate specie of Cr exhibiting toxic, genotoxic, and carcinogenic effects within cells, Cr(III) compounds themselves have been considered not only non-carcinogenic, but as an essential nutrient or supplement, and are provided in many dietary supplements [13]. The poor ability of Cr(III) to pass through cell membranes has been implicated in its low biological and toxicological activities, probably because it can form highly stable complexes with ligands [14]. Chromium(III) supplements such as Cr picolinate and Cr nicotinate have been widely used to enhance insulin function in regulating glucose, protein, and fat metabolism [15]. However, a number of recent studies have reported deleterious effects of Cr(III) supplementation. Moreover, Cr picolinate was reported to be harmful to humans and might have the potential to cause cancer [16]. Additionally, there are difficulties in performing experiments with Cr(III) due to its strong ligand binding. The lack of solid evidence of its essentiality in biological systems, raises concerns over its essentiality and the safety of ingestion of large quantities of Cr(III) by humans.

In this section, the essentiality of Cr(III) will be reviewed briefly. Then, recent studies regarding toxicological, carcinogenetic, genotoxic, mutagenetic, as well as epigenetic effects of Cr(III), in particular those of Cr picolinate, in the literature will also be discussed.

## BENEFICIAL EFFECTS OF Cr(III) AND QUESTIONS CONCERNED

A large body of literature has implicated Cr(III) as an essential element involved in the action of insulin as demonstrated in the studies of Cr deficiency in both experimental animals and humans. The role of Cr(III) in animals was reported about four decades ago in 1959 [17]. The study reported that Cr was required for maintaining normal glucose tolerance in rats [17]. Its essentiality in humans was reported later in 1977 [18]. A 40-year-old female patient who had received total parenteral nutrition (TPN) for more than 5 years developed severe diabetes. The signs included weight loss and hyperglycemia that was refractory to increased insulin dosing. These symptoms were ameliorated substantially following supplementing Cr to the patient for 2 weeks. In addition, other studies also showed that Cr was required for maintenance of normal glucose tolerance in humans [19–21]. In one case, a patient developed severe glucose intolerance, weight loss, and a metabolic encephalopathy-like confusional state after complete bowel resection and 5 months of TPN. Meanwhile, her serum Cr levels were at the lowest normal level. Supplementation of 150 μg of Cr per day reversed the glucose intolerance, reduced insulin requirements, and resulted in weight gain and the disappearance of encephalopathy [19]. Therefore, according to the fact of the low levels of Cr in the patients and their response to Cr supplementation, these studies suggested that Cr deficiency could arise in long-term TPN and Cr was a critical cofactor in the action of insulin. However, recent research has shown that it is rare for humans to develop Cr deficiency. Therefore, it is unlikely for healthy people to benefit from Cr supplementation [16].

Although it has been four decades since Cr was supplemented by patients with diabetes and anecdotal, the role of Cr supplementation to enhance insulin action is still controversial, and studies elucidating the mechanisms of the nutrient Cr at the molecular level are scarce. Among the limited research regarding the cellular and molecular mechanisms of Cr supplementation, deleterious effects of Cr have also been demonstrated.

## TOXICOLOGICAL AND CARCINOGENETIC EFFECTS OF Cr(III)

Chronic use of Cr picolinate has been reported to cause nephrotoxicity in humans if ingested in excess [22]. The patient, a 33-year-old woman, had ingested Cr picolinate 1200–2400 μg/d for 4–5 months to enhance weight loss. She had Cr plasma concentrations 2–3 times greater than the normal values and was presented with weight loss, anemia, thrombocytopenia, hemolysis, liver dysfunction (aminotransferase enzymes 15–20 times normal, total bilirubin 3 times normal), and renal failure (serum creatinine 5.3 mg/dL; blood urea nitrogen 152 mg/dL). All of these parameters returned to the normal values after she stopped consuming Cr picolinate for one year [22].

In addition, it has been reported that Cr picolinate consumption is associated with some acute physiological and behavioral impairment. For example, a clinical case demonstrated that Cr picolinate caused acute generalized exanthematous pustulosis in a 32-year-old man [23], and it caused systemic chronic dermatitis in a 35-year-old man [24]. In another case, a 35-year-old man suffered from progressively worsening episodes of cognitive, perceptual, and motor changes that interfered with his ability to drive a car after taking Cr picolinate [25].

Although Cr(VI) compounds were significantly more cytotoxic and mutagenic than Cr(III) compounds, both of them induced anchorage independence in a dose-dependent manner in cultured diploid human fibroblasts [26].

Chromium supplements have been shown to generate reactive oxygen species (ROS) in cells [27–29]. Reactive oxygen species are oxygen-containing and highly reactive molecules with unpaired electrons formed during oxidative metabolism. These species include the superoxide anion ($O_2^{\bullet -}$), the hydroxyl radicals ($^{\bullet}OH$), and hydrogen peroxide ($H_2O_2$). Considerable evidence has shown that ROS play an important role in the initiation of cellular injury which can lead to the development of cancer. Chromium picolinate had greater effects than Cr nicotinate in cultured macrophage J774A.1 cells [29]. In addition, an increase of lipid peroxidation and generation of DNA fragmentation were also found by both supplements [29]. Mitochondrial damage and apoptosis were observed when Chinese hamster ovary (CHO) cells were exposed to Cr picolinate [30]. In another comparative study of Cr picolinate and niacin-bound Cr(III), it was revealed that Cr picolinate produced significantly more oxidative stress and DNA damage [28]. Moreover, the study has implicated the potential toxicity of Cr picolinate in renal impairment, skin blisters and pustules, anemia, hemolysis, tissue edema, liver dysfunction; neuronal cell injury, impaired cognitive, perceptual and motor activity; enhanced production of hydroxyl radicals, chromosomal aberration, depletion of antioxidant enzymes, and DNA damage [28].

## GENOTOXIC AND MUTAGENETIC EFFECTS OF Cr(III)

Soluble Cr picolinate was found to produce chromosome damage 3 to 18-fold above control levels at doses ranging from 50 μM to 1.0 mM after 24 h treatment in CHO cells. Similarly, particulate Cr picolinate produced chromosome aberrations 4 and 16-fold above control levels at doses of 8.0 μg/cm$^2$ (corresponding to a 0.10 mM solublized dose) and 40 μg/cm$^2$ (0.50 mM), respectively [31]. Although picolinic acid was also

found to cause clastogenic damage, it was significantly less than that from Cr picolinate at equivalent doses. No detectable damage was observed when cells were exposed to Cr nicotinate, nicotinic acid, or $CrCl_3$ though [32].

The potential deleterious in vivo effects of Cr picolinate were also examined in *Drosophila melanogaster*. At levels of 260 μg Cr/kg food or less, Cr picolinate was found to lower the success rate of pupation and eclosion as well as to arrest development of pupae in a dose-dependent manner. Additionally, X-linked lethal analysis indicated that the supplement greatly enhanced the rate of appearance of lethal mutations and dominant female sterility. However, $CrCl_3$ failed to show the above effects [32].

At physiological relevant doses, Cr picolinate has been demonstrated to cause mutation at the hypoxanthine (guanine) phosphoribosyltransferase (*hprt*) locus of CHO cells. The mutation frequency was about 40-fold more than the untreated control and 4 times higher than $CrCl_3$-treated cells [33]. It also caused significant cell death [33]. It should be noted that exposures to Cr picolinate in these studies were using a water insoluble form, and attempts to solubilize it with acetone or DMSO may alter its mutagenic and clastogenic activities.

In a study investigating the metabolic fate of Cr(III) humic acid complexes and other Cr(III) organic complexes in mammalian systems, the results showed that a significant amount of Cr(III) was released (66% and 100%, respectively) and that *N*-1-methylpicotinamide was the primary organic metabolite from Cr picolinate in both human hepatocyte microsomes and primary cultures of chick hepatocytes. These data suggest that the populations of humans who are exposed to Cr(III) picolinate or other environmentally relevant organic Cr(III) complexes, (i.e. Cr(III) humic acid complexes), are potentially accumulating high levels of Cr(III) in cells. This intracellular accumulation of Cr(III) can result in the formation of covalent bonds between Cr(III) and DNA and/or other macromolecules, causing genotoxic effects [34].

Chromium(III) complexes have been shown to cause DNA strand breaks [27, 35]. Particularly, the mutagenicity of Cr complexes has been linked to their ability to unwind supercoiled DNA in an in vitro study [36].

## EPIGENETIC EFFECTS OF Cr(III)

By utilizing a mouse model (NIH Swiss), it has been shown that exposure of fathers to $CrCl_3$ for 2 weeks before mating can alter incidence of neoplastic and nonneoplastic changes in tissues of their offspring. Significant hypomethylation of the 45S ribosomal RNA (rRNA) gene was found in the sperm of the treated fathers. It has been known that the transcription of the 45S rRNA gene is regulated by rDNA methylation, and studies have indicated that abnormal expression of the gene in sperm is associated with neoplasia. The 45S rRNA is the precursor of 28S, 18S, and 5.8S rRNA, and the gene repeats hundreds of times in the mouse genome. About 40% of the gene is methylated in metabolically active cells in the mouse, and methylation-dependent transcriptional silencing of the gene can be inherited across generations. Differential methylation of the 45S rRNA gene has been observed in cancers including colon cancer, lung cancer, and Wilms' tumor. Differentiation of HL-60 leukemia cells is associated with marked decrease in the transcription of the gene. In contrast, estrogen-induced increase in rRNA

transcription correlates with pituitary tumor susceptibility in rats. Although the levels of rRNA in tissues of offspring from Cr(III)-treated fathers have not yet been monitored, this study has shown that the expression of various genes and the levels of serum hormones were altered in the offspring. For example, the levels of serum T3, the biologically active thyroid hormone, were elevated. So were those of its precursor, the thyroid hormone thyroxine T4. Accordingly, the expression of a number of hepotic genes, including those regulating metabolism, growth, intracellular signaling, transcription, and cytoskeleton, has been shown to be correlated with the increased levels of serum hormones. These changes may potentially contribute to enhanced growth rate and higher tumor risk. Consistent with these results, the study also demonstrated that both male and female offsprings of the Cr(III)-treated fathers were significantly heavier than those of the control fathers. Based upon these findings, it is conceivable that an epigenetic and/or gene expression–based mechanism may be involved in transgenerational carcinogenesis of Cr(III).

## CONCLUSIONS

Chromium(III) complexes have been used broadly as dietary supplements for decades. Although the investigation of the biochemical and nutritional properties of Cr(III) has been intensively undertaken since the 1960s, little has been achieved regarding its essentiality, function, or mechanisms of action. Given the evidence from the recent studies on the deleterious effects of Cr(III), especially those from Cr picolinate; the best-selling Cr supplement, more consideration should be taken into account on the safe usage of Cr(III).

## REFERENCES

[1] Vitale, R. J., Mussoline, G. R., and Rinehimer, K. A. (1997) Environmental monitoring of chromium in air, soil, and water. *Regul. Toxicol. Pharmacol. 26*, S80–S85.
[2] Fishbein, L. (1981) Sources, transport and alterations of metal compounds: An overview. I. Arsenic, beryllium, cadmium, chromium, and nickel. *Environ. Health Perspect. 40*, 43–64.
[3] IARC (1990) Chromium, Nickel and Welding. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 49*, 1–648.
[4] Arslan, P., Beltrame, M., and Tomasi, A. (1987) Intracellular chromium reduction. *Biochim. Biophys. Acta 931*, 10–15.
[5] Buttner, B., and Beyersmann, D. (1985) Modification of the erythrocyte anion carrier by chromate. *Xenobiotica 15*, 735–741.
[6] Mikalsen, A., Alexander, J., and Ryberg, D. (1989) Microsomal metabolism of hexavalent chromium. Inhibitory effect of oxygen and involvement of cytochrome P-450. *Chem. Biol. Interact. 69*, 175–192.
[7] Stearns, D. M., Kennedy, L. J., Courtney, K. D., Giangrande, P. H., Phieffer, L. S., and Wetterhahn, K. E. (1995) Reduction of Cr(VI) by ascorbate leads to Cr-DNA binding and DNA strand breaks in vitro. *Biochemistry 34*, 910–919.
[8] Zhitkovich, A., Voitkun, V., and Costa, M. (1995) Glutathione and free amino acids form stable complexes with DNA following exposure of intact mammalian cells to chromate. *Carcinogenesis. 16*, 907–913.

[9] Zhitkovich, A., Voitkun, V., and Costa, M. (1996) Formation of the amino acid-DNA complexes by hexavalent and trivalent chromium in vitro: importance of trivalent Cr and the phosphate group. *Biochemistry 35*, 7275–7282.
[10] Voitkun, V., Zhitkovich, A., and Costa, M. (1994) Complexing of amino acids to DNA by chromate in intact cells. *Environ. Health Perspect. 102 (Suppl. 3)*, 251–255.
[11] Agency for Toxic Substances and Disease Registry (2000) *Toxicological Profile for Chromium*, U. S. Public Health Service, U. S. Department of Health and Human Services, Atlanta, GA.
[12] U. S. Environmental Protection Agency (1983) *Health Assessment Document for Chromium*, EPA/600/8-83/014A, Environmental Assessment and Critieria Office, Office of Health and Environmental Assessment, Office of Research and Development, Research Triangle Park, NC.
[13] Anderson, R. A. (1981) Nutritional role of chromium. *Sci. Total Environ. 17*, 13–29.
[14] Zhitkovich, A. (2005) Importance of chromium-DNA adducts in mutagenicity and toxicity of Cr(VI). *Chem. Res. Toxicol. 18*, 3–11.
[15] Mertz, W. (1993) Chromium in human nutrition: a review. *J. Nutr. 123*, 626–633.
[16] Vincent, J. B. (2004) Recent developments in the biochemistry of chromium(III). *Biol. Trace Elem. Res. 99*, 1–16.
[17] Mertz, W., and Schwarz, K. (1959) Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. *Am. J. Physiol. 196*, 614–618.
[18] Jeejeebhoy, K. N., Chu, R., Marliss, E. B., Greenberg, G. R., and Bruce-Robertson, A. (1977) Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am. J. Clin. Nutr. 30*, 531–538.
[19] Freund, H., Atamian, S., and Fischer, J. E. (1979) Chromium deficiency during total parenteral nutrition. *JAMA 241*, 496–498.
[20] Anderson, R. A. (1995) Chromium and parenteral nutrition. *Nutrition 11(Suppl. 1)*, 83–86.
[21] Kimura, K. (1996) [Role of essential trace elements in the disturbance of carbohydrate metabolism]. *Nippon Rinsho 54*, 79–84.
[22] Cerulli, J., Grabe, D. W., Gauthier, I., Malone, M., and McGoldrick, M. D. (1998) Chromium picolinate toxicity. *Ann. Pharmacother. 32*, 428–431.
[23] Young, P. C., Turlansky, G. W., Bonner, M. W., and Benson, P. M. (1999) Acute generalized exanthematous pustulosis induced by chromium picolinate. *J. Am. Acad. Dermatol. 41*, 820–823.
[24] Fowler, J.F., Jr. (2000) Systemic contact dermatitis caused by oral chromium picolinate. *Cutis* 65, 116.
[25] Huszonek, J. (1993) Over-the-counter chromium picolinate. *Am. J. Psychiatry 150*, 1560–1561.
[26] Biedermann, K. A., and J. R. Landolph (1990) Role of valence state and solubility of chromium compounds on induction of cytotoxicity, mutagenesis, and anchorage independence in diploid human fibroblasts. *Cancer Res. 50*, 7835–7842.
[27] Speetjens, J. K., Collins, R. A., Vincent, J. B., and Woski, S. A. (1999) The nutritional supplement chromium(III) tris(picolinate) cleaves DNA. *Chem. Res. Toxicol. 12*, 483–487.
[28] Bagchi, D., Stohs, S. J., Downs, B. W., Bagchi, M., and Preuss, H. G. (2002) Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology 180*, 5–22.
[29] Bagchi, D., Bagchi, M., Balmoori, J., Ye, X., and Stohs, S. J. (1997) Comparative induction of oxidative stress in cultured J774A.1 macrophage cells by chromium picolinate and chromium nicotinate. *Res. Commun. Mol. Pathol. Pharmacol. 97*, 335–346.
[30] Manygoats, K. R., Yazzie, M., and Stearns, D. M. (2002) Ultrastructural damage in chromium picolinate-treated cells: A TEM study. *J. Biol. Inorg. Chem.7*, 791–798.

[31] Stearns, D. M., Wise, J. P., Jr., Patierno, S. R., and Wetterhahn, K. E. (1995) Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. *FASEB J. 9*, 1643–1648.

[32] Hepburn, D. D. D., Xiao, J., Bindom, S., Vincent, J. B., and O'Donnell, J. (2003) Nutritional supplement chromium picolinate causes sterility and lethal mutations in *Drosophila melanogaster. Proc. Natl. Acad. Sci., USA 100*, 3766–3671.

[33] Stearns, D. M., Silviera, S. M., Wolf, K. K., and Luke, A. M. (2002) Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. *Mutat. Res. 513*, 135–142.

[34] Kareus, S. A., Kelley, C., Walton, H. S., and Sinclair, P. R. (2001) *Release of Cr(III) from Cr(III) picolinate upon metabolic activation. J. Hazard. Mater. 84*, 163–174.

[35] Sugden, K. D., Geer, R. D., and Rogers, S. J. (1992) Oxygen radical-mediated DNA damage by redox-active Cr(III) complexes. *Biochemistry 31*, 11626–11631.

[36] Blankert, S. A., Coryell, V. H., Picard, B. T., Wolf, K. K., Lomas, R. E., and Stearns, D. M. (2003) Characterization of nonmutagenic Cr(III)-DNA interactions. *Chem. Res. Toxicol. 16*, 847–854.

This page intentionally left blank

*Chapter 13*

# Summary: The clinical and nutritional importance of chromium – Still debated after 50 years of research

**Forrest H. Nielsen***

USDA, ARS, Grand Forks Human Nutrition Research Center Grand Forks, ND 58202-9034

## INTRODUCTION

The first suggestion that chromium may be biologically active appeared in 1954 when it was reported that chromium enhanced the synthesis of cholesterol and fatty acids from acetate in rat liver [1]. In 1959, trivalent chromium was reported to be the glucose tolerance factor that alleviated the 1955 discovery of impaired glucose tolerance in rats fed torula yeast-sucrose diets [2, 3]. Shortly thereafter, the glucose tolerance factor, or GTF, evolved into a speculated organic form of chromium. Several reports appeared in the 1960s and 1970s suggesting that chromium as GTF normalized insulin responses and improved glucose tolerance in some individuals with impaired glucose tolerance or mild diabetes [4]. Despite these suggestive findings, flaws in the early studies of chromium nutrition described by Vincent and Stallings (Chapter 1) inhibited the acceptance of chromium as an essential nutrient until 1977. In that year it was reported that signs of chromium deficiency were found in a patient receiving total parenteral nutrition [5]. This report apparently was a major factor for the establishment of an estimated safe and adequate daily dietary intake (ESADDI) for chromium by the Food and Nutrition Board in 1980 [6]. Shortly thereafter, however, the nutritional essentiality of chromium was questioned anew when it was found that chromium analyses before 1980 were not valid and repeated efforts to definitively characterize a chromium-containing GTF were unsuccessful, which raised doubts about its existence. However, the debate about the clinical and nutritional importance of chromium did not become heated until after 1989. This is when it was reported that chromium in the form of chromium picolinate increased lean muscle mass and decreased body fat in young men participating in a weight-training

* The opinions expressed in this article are those of Forrest H. Nielsen; they do not represent or should not be construed as the official position or policy of the US Department of Agriculture.
The USDA, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer, and all agency services are available without discrimination.

The Nutritional Biochemistry of Chromium(III)
*John B. Vincent (Editor)*
ISBN: 0-444-53071-1

program [7]. This led to claims that chromium picolinate stimulated weight loss without altering food intake or exercise levels. Another report that provoked the debate was one describing remarkable decreases in blood glucose and glycated hemoglobin found in 180 diabetic Chinese subjects after being supplemented with 1000 μg Cr/d as chromium picolinate for 4 months [8]. These claims for chromium were immediately questioned and reports appeared stating difficulty in confirming these beneficial actions of chromium (described below). These reports also focused attention on the question of whether chromium should be accepted as an essential nutrient. As indicated by Vincent and Stallings (Chapter 1) and Stearns (Chapter 3), credible evidence to support essentiality is limited. Whether a specific biochemical function for chromium has been identified is still questioned, and a clear set of consistent deficiency signs has not been established for higher animals or humans.

The divergent interpretations of the findings regarding the clinical, nutritional, and toxicological attributes of chromium in the preceding chapters illustrate that chromium is a prime example of scientists championing a hypothesis, polarized views, unproven claims, and suspicions about commercial bias and conflict of interest in nutrition. Readers should carefully evaluate the material of each chapter in this book to formulate their own conclusions about the clinical, nutritional, and toxicological importance of chromium. As a scientist who has not performed research in the chromium field, but has had numerous friendly debates with those who have and has routinely read research articles about chromium, my summary of the preceding chapters and personal opinions about the status of chromium may provide some help in formulating an opinion about whether chromium is a bioactive beneficial if not essential element, or an element of inconsequence or toxicological concern for human health.

## CHROMIUM(III) AS A NUTRIENT

Before the 1960s, nutrients accepted as essential for an organism were those whose dietary deficiency resulted in death, interrupted the life cycle, or resulted in a failure to grow. During the 1960s and 1970s, the definition of essentiality was modified for mineral elements that could not be fed at dietary concentrations low enough to cause death or interrupt the life cycle (interfere with growth, development, or maturation such that procreation is prevented). Most definitions included one or more of the following criteria [9]. (1) The element must react with biological material to form chelates. (2) The element must be ubiquitous in seawater and earth's crust. (3) The element must be present in significant quantity in animals. (4) The element should be toxic to animals only at relatively high intakes in comparison to nutritional intakes. (5) Homeostatic mechanisms must exist for the element so that it is maintained in the body in a rather consistent amount during short-term variations in intake. (6) Finally, and most importantly, a dietary deficiency must consistently result in a reduction of a biological function from optimal that is preventable or reversible by physiological amounts of the element. Physiological amounts are usually defined as amounts usually found in biological material. In the 1960s and 1970s, chromium appeared to fulfill all these criteria. The biological function that was supposedly consistently impaired was glucose metabolism. Thus, chromium was generally accepted as an essential nutrient. This acceptance is illustrated by the 1989

report of the Food and Nutrition Board [10] in which it was stated, "Trivalent chromium is required for maintaining normal glucose metabolism in laboratory animals; it acts as a cofactor for insulin."

However, in the 1990s, establishing essentiality on the basis of the criteria listed above began to receive resistance when many elements were suggested to be essential based on some small change in a physiological or biochemical variable in an experimental animal model supposedly fed a diet deficient in a specific element. Doubts were voiced about whether some of the changes were a result of low intakes causing suboptimal functions. The possibility was raised that some of the changes may have been manifestations of a supra- nutritional or pharmacological action; that is, the high intake of an element was having a therapeutic action such as alleviating the deficiency signs of another nutrient, or enhancing a biochemical process or improving a biological structure (e.g., fluoride as anti-carious agent). As a result of these concerns, if the lack of an element cannot be shown to cause death or interrupt the life cycle, at present, an element is not generally accepted as essential unless it has a clearly defined biochemical function. The change in the definition of essentiality apparently has altered the essentiality status of chromium. The Food and Nutrition Board in its latest discussion of chromium [11] states that "chromium potentiates the action of insulin in vivo and in vitro," "a number of studies have demonstrated beneficial effects of chromium on circulating glucose, insulin, and lipids in a variety of human subjects and animal species," and "not all reports of supplementation are positive." The words "required" and "essential" are not used for chromium.

There are good reasons for chromium apparently losing its designation as an established essential nutrient. A chromium deficiency has not been induced in any animal species that caused death or interrupted the life cycle. Vincent and Stallings (Chapter 1), Stearns (Chapter 3), and Vincent and Bennett (Chapter 7) indicate that a clearly defined biochemical function has not been found for chromium (described in more detail below). Studies of chromium essentiality subsequent to 1985 when improved analytical methods for chromium appeared indicate that chromium is even unable to meet the requirements of the old definition of essentiality. Vincent and Stallings (Chapter 1) and Stearns (Chapter 3) disclose that reports of apparent chromium deficiency do not describe a *consistent* reduction in a biological function from optimal that is prevented by *physiological amounts* of chromium. As indicated by Vincent and Stallings (Chapter 1), the first animal studies using torula yeast or seed rye diets may have not been low in chromium, may have contained undefined dietary stressors, and provided chromium supplements to controls that resulted in chromium intakes over 100 times the amount that may be considered physiological. Thus, the effects on glucose metabolism and circulating cholesterol in the first animal experiments may have been an indication of a pharmacologic action of chromium. In the later experiments in which the chromium contents of supposedly deficient diets were determined, consistent deficient signs were not reported. Even a study that was very meticulous in controlling chromium intake and used dietary stressors [12] apparently had difficulty in inducing signs of a chromium deficiency. This experiment did not show a marked effect of chromium on glucose tolerance of rats, often considered the primary indicator of a requirement for chromium. Vincent and Stallings (Chapter 1) and Stearns (Chapter 3) also describe the rather inconsistent symptoms of five patients on TPN that responded to chromium supplementation.

The assumed chromium deficiency symptoms affected by chromium supplementation of impaired glucose utilization, glucose intolerance, neuropathy, or encephalopathy were not consistently found in all patients. Also, the amount of chromium supplemented to the TPN patients that did not have to overcome the gastrointestinal barrier to absorption probably could be considered pharmacologic.

To conclude, chromium at present does not fulfill any definition of essentiality. However, this lack does not mean chromium is not essential, and may be found so in the future.

## CHROMIUM AS A NUTRITIONAL SUPPLEMENT

Recently a paradigm has emerged in which recommendations are being made for intakes of dietary substances that are not considered essential because of their apparent health benefits, for example omega-3 fatty acids for cardiovascular health and fiber for gastrointestinal health. Also, dietary intake recommendations for some essential nutrients are being made or being suggested that far exceed those required to prevent deficiency pathology because of apparent health benefits, for example calcium to prevent bone loss and selenium for the prevention of some types of cancer. Terms such as functional foods, nutraceuticals, and phytonutrients are prevalent in discussions of diet in the promotion of health not involving the prevention of deficiency pathology. Thus, the lack of evidence for chromium essentiality does not preclude the concept that chromium is a bioactive element, and when supplemented in higher than physiological (supra nutritional) amounts may have beneficial effects. Numerous claims for such beneficial effects provided by nutritional supplements are described by Lukaski (Chapter 4)) and Lindemann (Chapter 5).

Among the claims for supra-nutritional effects of chromium provided by supplements for humans are weight reduction, body composition change (increased muscle and decreased fat mass), and enhanced physical performance. Lukaski (Chapter 4) describes well-controlled studies that show intakes of 200 μg Cr/day compared to relatively low intakes of chromium had no discernable effect on body composition and muscular strength. Still it may be too soon to dismiss chromium as an ergogenic aid for athletes because one study has suggested a higher dose (400 μg/day) with long-term (24 week) very high intensity aerobic exercise (a stressor that may increase chromium use, see below) may elicit body fat loss and muscle gain [13]. Lukaski (Chapter 4) also evaluates studies determining the effect of chromium on weight reduction. Studies finding chromium-inducing weight reduction were clouded by lack of control of chromium and energy intakes and the use of an unusual indicator (body composition improvement index) of weight reduction. Well-controlled studies using recognized status assessment measures have found that supra-nutritional (200–400 μg/d) chromium supplementation had negligible effects on weight reduction or maintenance of weight loss.

Lindemann (Chapter 5) describes numerous studies by several research groups that indicate domestic animal responses to nutritional supplements providing organic forms of chromium (chromium picolinate, chromium propionate, chromium methionine, and chromium yeast) have been more supportive than human responses to the concept that chromium has beneficial effects in supra-nutritional amounts. In swine, organic forms of

chromium supplying 200 μg Cr/kg to diets containing significant amounts of chromium often improved muscling and feed efficiency when protein intake met recommendations; however, feeding excess protein diminished or eliminated the response. Supplementing the diet of sows with 200–600 μg Cr/kg has been shown to increase average litter size with the effect more apparent when performance is compromised. Reproduction in swine is considered a diabetogenic event. Lindemann (Chapter 5) has presented findings suggesting that the basis for organic forms of chromium increasing litter size was through an effect on insulin sensitivity. Supplementing 500–1000 μg of chromium in organic forms was found to decrease morbidity and increase feed efficiency in calves stressed by being placed from a grazing situation to a feedlot. Chromium supplementation was of most benefit to calves that had the greatest cumulative degree of stress. Chromium picolinate supplementation (3500 μg/d) has been found to decrease the incidence of retained placenta in dairy cows. Broiler (meat chicken) mortality is decreased by organic chromium supplementation, especially under conditions in which mortality is high (apparently from cumulative stress caused by crowding, disease challenge, heat, etc.). In horses under exercise stress, chromium supplementation as chromium yeast apparently improved energy utilization. Thus, supra-nutritional supplementation of farm livestock has a variety of beneficial effects. Most benefits seem to occur when the animal is exposed to some form of stressor.

Lindemann (Chapter 5) has interpreted the findings with farm livestock as possible evidence for chromium essentiality. He suggests that under stress situations, there is an increased need for chromium that is not supplied by the diet. This occurs even if the diet contains significant amounts of chromium because the chromium is not very bioavailable. Supplementation with bioavailable organic forms of chromium is effective in alleviating or preventing the conditional chromium deficiency. Thus, his interpretation would fit my definition of nutritionally beneficial or conditionally essential [14]. However, another interpretation of the findings could be that the organic forms of chromium were having a pharmacological beneficial effect [14]. Further discussion of the pharmacologic or therapeutic actions of chromium is presented below. Regardless of whether its actions are essential, conditionally essential, nutritionally beneficial, or pharmacologically beneficial, chromium can be considered a bioactive beneficial mineral element, especially in some organic forms, based on responses of both animals and humans to nutritional supplements.

## BIOCHEMICAL ROLE(S) FOR Cr(III)

Discussions by Vincent and Stallings (Chapter 1), Stearns (Chapter 3), Feng (Chapter 6), and Vincent and Bennett (Chapter 7) evince that a biochemical role for chromium has not been definitively established. Stearns (Chapter 3) and Vincent and Bennett (Chapter 7) discuss the lack of experimental evidence that has eliminated the "glucose tolerance factor" as a biochemical role for chromium. Also, Vincent and Bennett (Chapter 7) have provided reasonable justifications for not considering increased insulin receptor number or chromate inhibition of phosphotyrosine phosphatase as mechanisms through which chromium has positive effects on insulin action or glucose metabolism.

Feng (Chapter 6) presents current knowledge about the metabolism of chromium which may suggest a physiological or essential function for chromium. Substantial

evidence exists indicating that transferrin is a major in vivo transport molecule for chromium. Both Stearns (Chapter 3) and Feng (Chapter 6) suggest that chromium may have an effect on circulating insulin and glucose through an interaction with iron through competitive binding to transferrin. Feng (Chapter 6) indicates that epidemiologic studies have repeatedly shown a positive correlation between concentrations of serum ferritin (iron storage molecule) and concentrations of fasting glucose, insulin, and glycosylated hemoglobin. Thus, the reason supra-nutritional amounts of chromium has been found to affect these metabolites may be through reducing the in vivo presence of iron. Although this may be a mechanism through which chromium has beneficial effects on glucose metabolism, it would not be considered an essential biochemical role for the element.

Chromium homeostasis apparently is maintained through urinary excretion and limited absorption. Feng (Chapter 6) presents evidence that absorbed chromium is transferred to a low-molecular-weight complex (LMWCr) for excretion in the urine. The extent to which LMWCr, often called chromodulin, occurs in tissues has not been firmly established. Although early studies described by Feng (Chapter 6) suggested that the predominant form of chromium in tissues was in the LMWCr form, later studies indicate that this is not the case. Although chromodulin may not be the predominant form of chromium in tissue such as liver, it has elicited much discussion as a possible molecule through which chromium has an essential biochemical role beyond that of an excretory molecule.

Feng (Chapter 6) indicates that chromium transfers from the blood compartment to insulin-sensitive tissues. Feng (Chapter 6) and Vincent and Bennett (Chapter 7) discuss findings showing that insulin increases the transfer of intravenous-injected chromium-transferrin complex from blood to tissues, especially liver and kidney; this increase preceded an increase in both tissue and urinary LMWCr. This suggests that the stimulation of insulin release promotes the movement of chromium from transferrin or some other high-molecular-weight complex to an LMWCr complex, or chromodulin, to perform a biochemical function, and after completing its function, the chromodulin is excreted. It should be noted, however, that Stearns (Chapter 3) suggests the increase in excretion of chromodulin after a relatively high dose of injected chromium-transferrin complex may be indicating that LMWCr or chromodulin is just involved in chromium homeostasis or the removal of excessive chromium in the body.

Vincent and Bennett (Chapter 7) describe chromodulin as a naturally occurring oligopeptide composed of glycine, cysteine, aspartate, and glutamate whose sequence has not been determined. Chromodulin tightly binds four chromium ions. The oligopeptide is maintained in the soluble portion and the nucleus of liver cells in the apo form. Thus, an in vivo or in vitro chromium-loading step is required to isolate the oligopeptide as holo-chromodulin. In vitro studies using isolated rat adipocytes have shown that holo-chromodulin amplifies the tyrosine kinase activity of insulin-activated insulin receptor; apo-chromodulin does not amplify the kinase activity. Vincent and Bennett (Chapter 7) have provided evidence that the ability of chromodulin to enhance the stimulation by insulin of the insulin-dependent protein tyrosine kinase activity of insulin receptor is specific to chromium and directly dependent on the chromium content of chromodulin. Based mainly on these in vitro results, chromodulin has been hypothesized to function as part of a unique autoamplification system for insulin signaling; this system is described by Vincent and Bennett (Chapter 7). This hypothesis remains to be experimentally

verified directly. As stated by Vincent and Bennett (Chapter 7), it needs to be established that chromodulin binds to the insulin receptor and that this interaction affects insulin receptor kinase activity in vivo. Interestingly, rats injected intravenously daily with 20 μg chromium as chromodulin for 12 weeks did not show any marked changes in glucose metabolism.

Although it very tempting to conclude that a biochemical role for chromium as an LMWCr complex, or chromodulin, has been found, it is apparent that further work is necessary before this conclusion can be reached. Thus, it cannot be stated that an essential biochemical function for chromium has been definitively established.

## CHROMIUM(III) AS A THERAPEUTIC AGENT

My definition of pharmacologically beneficial [14] includes the concept that a high dietary intake (i.e., 50–100 times the normal intake) of a nutrient or dietary substance alters a biochemical pathway(s) or biological structure(s) in a therapeutic way. The Food and Nutrition Board [15] has stated that for pharmacological effects to occur, doses greatly exceeding the amount of a nutrient present in foods are usually needed to obtain a therapeutic response; the pharmacological action often is different than the physiological action of the nutrient; and the chemical analogues of the nutrient that are often most effective pharmacologically may have little or no nutritional activity. Based on the dosages and the forms of chromium that are most effective in inducing beneficial effects, many findings reported as evidence for the essentiality of chromium may be the result of pharmacologic actions.

As indicated above, the beneficial effects of organic forms of chromium in farm animals could be interpreted as being results of pharmacologic actions. Vincent and Bennett (Chapter 7) review findings from the administration of another organic form of chromium, the propionate biomimetic of chromodulin, to experimental animals. Like chromodulin, the propionate mimetic activated insulin receptor kinase activity in vitro. However, unlike chromodulin which had very little effect, the propionate mimetic had striking effects in vivo; 20 μg/kg body weight daily for 12 weeks administered intravenously lowered plasma triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol in both healthy and Zucker obese rats, but not in rats with streptozotocin-induced diabetes. Oral gavage of 250, 500, or 1000 μg Cr/kg body weight as the propionate mimetic also lowered fasting plasma insulin, triglycerides, total cholesterol, and LDL-cholesterol, but did not affect plasma glucose or HDL-cholesterol in healthy rats. Other forms of chromium such as chromium chloride and chromium picolinate at these doses have not been found to affect these variables. The lowering of plasma insulin without affecting plasma glucose suggests increased insulin sensitivity. In Zucker obese rats and ZDF rats (a genetic model for type 2 diabetes), 1000 μg Cr/kg body weight as the propionate mimetic also lowered plasma insulin and glycated hemoglobin. The high dosages used and the effects on variables not affected by other forms of chromium suggest that this propionate mimetic of chromium was having pharmacologic effects in healthy rats and therapeutic effects in diabetic rat models; the therapeutic effect being like that of other drugs, such as pioglitazone – that is, the mimetic increases insulin sensitivity to alleviate diabetic pathology. This does not preclude the possibility that

the pharmacologic effect is mediated through an elevation in the formation of chromodulin. If this is the case, then chromium may be considered a conditional essential trace element. A conditional essential nutrient may be defined as one that is ordinarily not required but under certain pathological or stressor conditions it is used to maintain health.

Based on the amounts and the nutritional or health status of experimental subjects, another organic form of chromium, niacin-bound chromium, apparently has beneficial pharmacologic or therapeutic effects. Zafra-Stone et al. (Chapter 8) lists numerous studies with findings showing that niacin-bound chromium in high concentrations lowers blood pressure and cholesterol. In many of these studies, the effect was apparent when the some form of stressor was present, or high amounts of niacin-bound chromium were given in combination with some other dietary factor. This includes the use of lipogenic diets for cholesterol effects and sugar-induced hypertension in blood pressure studies, and the use of aged Zucker fatty rats or spontaneously hypertensive rats for both cholesterol and blood pressure effects. In both blood pressure and cholesterol reduction studies, the combination of high amounts of niacin-bound chromium (i.e., 5 mg/kg diet) with zinc methionine or grape seed proanthocyanidin over a prolonged period resulted in the most significant reductions. These findings suggest that the niacin-bound chromium was bioactive but was not overcoming a chromium deficiency.

The findings with organic forms of chromium in farm animals and with the propionate mimetic and niacin-bound chromium in rats suggest that high dosages of chromium in organic forms given to humans may be acting pharmacologically or therapeutically to affect insulin sensitivity and lipid metabolism. This judgment is supported by the review of Cefalu (Chapter 9). He points out that whether chromium supplementation has a beneficial effect depends on the dose and form of the chromium supplement, the length of time of supplementation, and the health of the subject taking the supplement. Trials that demonstrate negative effects of chromium on variables associated with insulin sensitivity or improved glucose metabolism have used relatively low doses of chromium ($\leq$250 μg/d), used low bioavailable forms of chromium (i.e., $CrCl_3$), or evaluated healthy non-diabetic people. Trials using higher doses of chromium supplementation (often 1000 μg/d), especially in the form of chromium picolinate which has relatively good bioavailablity, in patients with type 1, type 2, gestational, or steroid-induced diabetes often had favorable effects on variables assessing carbohydrate metabolism. The conditions under which chromium had positive effects indicate that the beneficial or therapeutic actions of chromium most likely were not the result of the supplementation alleviating a chromium deficiency. However, the therapeutic findings do not preclude the possibility that a low chromium intake contributed to becoming diabetic upon exposure to stessors that cause this disorder. In other words, as stated above, chromium may be conditionally essential or nutritionally beneficial when an experimental animal or human is exposed to a stressor causing metabolic changes that would be improved by increased insulin sensitivity. Support for this possibility is that several large epidemiologic studies have suggested a relationship between chromium status and cardiovascular disease. For example, the risk of myocardial infarction was reported to be significantly lower in men with the highest quintile for toenail chromium concentration, but the relationship was significant only for subjects with a body mass index $\leq$25 kg/m$^2$.

## TOXICOLOGICAL EFFECTS OF Cr(III)

In addition to essentiality, the toxicity of Cr(III) also is a controversial subject. This controversy is fueled by in vitro chemical studies, cell culture toxicity determinations, and administration of chromium complexes to animal models in a manner that does not require them to overcome the gastrointestinal barrier. These studies show that high amounts of some forms of chromium may cause oxidative stress and DNA damage. In contrast, dietary ingestion of high amounts of various forms of Cr(III) has not been found to induce any marked signs of toxicity. For example, Schroeder et al [16]. fed mice 5000 μg Cr/mL drinking water as chromium acetate for 17 months without any apparent toxicity effects. Instead they found that this high intake of chromium increased growth and decreased mortality. Anderson et al. [17] observed no toxic effects in rats fed 100,000 μg Cr/kg diet as chromium picolinate for 24 weeks. Unfortunately, no indicator of oxidative stress was determined in this study. In contrast, Hepburn et al. [18] found that rats intravenously injected daily with 5 μg of chromium as a picolinate complex for 60 days excreted elevated amounts of 8-hydroxydeoxyguanosine (a product of oxidative DNA damage) in urine, and increased amounts of peroxidized lipids in tissues. Stearns (Chapter 11) describes studies which show relatively high amounts of chromium picolinate are clastogenic, mutagenic, and genotoxic in some, but not all, standard assays using cultured cells that are used to predict carcinogens.

Based on the redox chemistry of chromium, Levina et al. (Chapter 11) describe possible mechanisms through which Cr(III) may be pro-oxidant and toxic. Levina et al. (Chapter 11) and Ke and Costa (Chapter 12) indicate that Cr(III) is unlikely to be toxic because its complexes with oxygen-based ligands are usually electrochemically inactive, and have poor ability to cross cell membranes. They also present evidence that direct reactions with DNA are unlikely for biologically relevant Cr(III) complexes. However, in the presence of biological oxidants, Cr(III) as hydrolyzed $CrCl_3$ or organic complexes (i.e., chromium picolinate) may be converted into Cr(VI). Chromium(VI) can react with cellular reductants to produce potentially DNA-damaging Cr(V/IV). Chromium(V/IV)-containing oxygen molecules are reactive and can complex with DNA. After forming the complex, Cr(V/IV) can be reconverted into Cr(III) and lead to the formation of protein-Cr(III)-DNA and DNA-Cr(III)-DNA crosslinks, which are highly stable and strongly disruptive to the transcription process because of the kinetic inertness of Cr(III). Also, the highly reactive Cr(V/IV) can cause oxidative damage to DNA. Chromium(V/IV) reactive species have been implicated as the main, if not ultimate, cause of Cr(VI)-induced genotoxicity and carcinogenicity. The formation of Cr(II) from Cr(III), particularly in the picolinate form, by biological reductants also has been suggested to result in reactive hydroxyl generation that can damage DNA [19].

If one of the hypothesized mechanisms described above is the basis for Cr(III) toxicity that results in the genotoxicity and mutagenicity described by Stearns (Chapter 10) found with cultured cells, the question becomes "what is the oral dosage of Cr(III) compounds needed to result in the formation of enough reactive forms of chromium to be of toxicological concern?" Vincent and Bennett (Chapter 7) indicate that the amount of chromate formed in vivo is likely to be very small when chromium is ingested in an amount commonly found in commercial supplements. Findings by Hepburn and Vincent [20] indicate that chromium picolinate is degraded and excreted quickly in vivo.

A single acute oral dose of 895,000 μg Cr(III)/kg to rats, as aqueous $CrCl_3$, causes increases in several markers of oxidative stress [21], but 5000 μg Cr(III)/mL water as chromium acetate fed to mice for 17 months did not have any apparent toxic effects [16]. These findings indicate that the amount of orally ingested Cr(III) has to be relatively high to cause an oxidative stress that the body cannot handle and thus result in toxicological consequences. However, if Cr(III) is made more readily available for oxidation by direct administration to cells or intravenous injection in animals, amounts closer to physiological may induce free radicals that have pathological consequences. For example, daily injection of chromium picolinate for 60 days into rats in amounts about 20 times that entering the circulation in humans consuming commercial supplements (on a per kg body weight basis) significantly increased the urinary excretion of 8-hydroxydeoxyguanosine, a product of oxidative DNA damage [18]. When one closely examines the dosages and methods of administration used, an impression is obtained that Cr(III) ingestion in amounts found in diets, drinking water, and one-a-day type of multivitamin mineral supplements can be consumed without fear of toxic consequences. Supplements supplying up to 1000 μg Cr(III) in various forms found in the marketplace are also most likely safe. However, chromium is the same as any other mineral element in that the dose is the poison. The question that remains to be determined is the concentration at which the various forms of orally ingested Cr(III) become of toxic concern because homeostatic mechanisms are unable to prevent chromium accumulation in high enough quantity in cells that will allow chemical reactions that can cause non-repairable damage to occur.

## CONCLUSION

Chromium should not be classified as an essential element because it is unable to meet any definition of essentiality. A clearly defined function has not been established for chromium. Neither has a chromium deficiency been induced in any animal species that causes death or interrupts the life cycle. Chromium is also unable to fulfill the older criteria of essentiality because a consistent reduction in a biological function from optimal that is prevented by physiological amounts of chromium has not been conclusively demonstrated. This inability to fulfill the requirements to be classified as essential does not preclude the possibility that chromium may be found essential in the future. However, research to date indicates that if chromium is essential, its requirement is extremely small. Based on the difficulty in producing signs of chromium deficiency in animals, adequate intake levels of 20–25 μg/d set for women and 30–35 μg/d set for men [11] should fulfill any possible chromium requirement.

Because chromium in organic forms has been shown to have beneficial effects mostly when an animal or human is exposed to a stressor that responds to enhanced insulin sensitivity or action, chromium may receive consideration for the classification as a conditionally essential nutrient. However, the term "conditionally essential" is usually applied to organic substances (i.e., glutamine, carnitine) with known biochemical functions and normally synthesized by the body, which under certain situations are not produced in sufficient quantities such that an endogenous source is needed. Chromium, of course, is not synthesized by the body, but LMWCr apparently is. It may be hypothesized that under normal conditions the need for insulin amplification is minimal, but under

stressor conditions that would benefit from insulin amplification, an increased intake of chromium – especially in the LMWCr (chromodulin) form, LMWCr mimetic form, or in a form that can be converted quickly into LMWCr – is needed to prevent pathological consequences. Still, conditional essentiality does not apply well to mineral elements in any form, and thus it is difficult to classify chromium as such.

Based on the information provided by the preceding reviews, the best classification for chromium is that it is a nutritionally or pharmacologically beneficial element. That is, chromium in supra-nutritional amounts alters biochemical pathway(s) or biological structure(s) in a pathologic preventive or therapeutic manner. The biochemical system apparently primarily affected by chromium is insulin sensitivity or action. This means that chromium may be in the same class as other substances provided in supra-nutritional amounts for health benefits, for example increased intakes of selenium, vitamin C, vitamin E, and caroteonids to alleviate oxidative stress and reduce the risk of chronic degenerative disease and cancer.

Chromium(III) compounds as provided by nutritional supplements in the marketplace have a relatively low order of toxicity when ingested. Animal and human studies suggest that long-term supplemental intakes of 200 μg/day, and short-term intakes (several months) between 200 and 1000 μg/day are safe. Further studies most likely will establish that all Cr(III) supplements in the marketplace at present are safe to consume in a 1000 μg/day quantity for therapeutic reasons. Chromium supplementation may be therapeutic or useful as an adjunct treatment for some cases of type 2 diabetes or other disorders caused by insulin insensitivity.

Because most healthy people do not respond to chromium supplementation, including exhibiting weight loss and improved body composition and strength, ingesting supplements that provide high amounts of chromium (>200 μg/day) for most people is not justifiable. Intakes near the adequate intakes set by the Food and Nutrition Board [11] should assure health and well-being for most people. The best and most enjoyable way of consuming healthful amounts of chromium is by eating a varied diet incorporating foods and beverages that are good sources of chromium. Some good sources of chromium are organ meats such as liver, whole grain products including some ready-to-eat bran-containing cereals, pulses such as dried beans, some vegetables including broccoli and mushrooms, spices, and beer. Although diet is the best source of chromium, some individuals may insist upon taking a supplement for "insurance" or "peace of mind." These people should be assured that a separate chromium supplement is unnecessary; a multivitamin-mineral supplement containing <200 μg of chromium will do.

## REFERENCES

[1] Curran, G. L. (1954) Effect of certain transition group elements on hepatic synthesis of cholesterol in the rat. *J. Biol. Chem. 210,* 765–770.

[2] Mertz, W., and Schwarz, K. (1955) Impaired intravenous glucose tolerance as an early sign of dietary necrotic liver degeneration. *Arch. Biochem. Biophys. 58*, 504–506.

[3] Schwarz, K., and Mertz, W. (1959) Chromium (III) and glucose tolerance factor. *Arch. Biochem. Biophys. 85*, 292–295.

[4] Mertz, W. (1979) Chromium – an overview in *Chromium in Nutrition and Metabolism (Dev. Nutr. Metab. 2)* Shapcott, D, and Hubert, I., eds.), pp. 1–14, Elsevier/North Holland Biomedical Press.

[5] Jeejeebhoy, K. N., Chu, R. C., Marliss, E. B., Greenberg, G. R., and Bruce-Robertson, A. (1977) Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am. J. Clin. Nutr. 30*, 531–538.
[6] Food and Nutrition Board, National Research Council (1980) *Recommended Dietary Allowances, 9th Revised Edition*, National Academy Press, Washington, DC.
[7] Evans, G. W. (1989) The effect of chromium picolinate on insulin controlled parameters in humans. *Int. J. Biosoc. Med. Res. 11*, 163–180.
[8] Anderson, R. A., Cheng, N. C., Bryden, N. A., Polansky, M. M., Cheng, N., Chi, J., and Feng, J. (1997) Elevated levels of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes 46*, 1786–1791.
[9] Nielsen, F. H. (1998) The justification for providing dietary guidance for the nutritional intake of boron. *Biol. Trace Elem. Res. 66*, 319–330.
[10] Food and Nutrition Board, National Research Council (1989) *Recommended Dietary Allowances, 10th edition,* National Academy Press, Washington, DC.
[11] Food and Nutrition Board, National Institute of Medicine (2002) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.* National Academy Press, Washington, DC.
[12] Striffler, J. S., Law, J. S., Polansky, M. M., Bhathena, S. J., and Anderson, R. A. (1995) Chromium improves insulin response to glucose in rats. *Metabolism 44*, 1314–1320.
[13] Bulbulian, R., Pringle, D. D., and Liddy, M. S. (1996) Chromium picolinate supplementation in male and female swimmers. *Med. Sci. Sports Exerc. 28*, Suppl., S111.
[14] Nielsen, F. H. (2000) Importance of making dietary recommendations for elements designated as nutritionally beneficial, pharmacologically beneficial, or conditionally essential. *J. Trace Elem. Exp. Med. 13*, 113–129.
[15] Food and Nutrition Board, Institute of Medicine. (1994) *How Should the Recommended Dietary Allowances be Revised?* National Academy Press, Washington, DC.
[16] Schroeder, H. A., Vinton, W. H., Jr., and Balassa, J. J. (1963) Effect of chromium, cadmium, and other trace elements on the growth and survival of mice. *J. Nutr. 80*, 39–47.
[17] Anderson, R. A., Bryden, N. A., and Polansky, M. M. (1997) Lack of toxicity of chromium chloride and chromium picolinate in rats. *J. Amer. Coll. Nutr. 16*, 273–279.
[18] Hepburn, D. D. D., Burney, J. M., Woski, S. A., and Vincent, J. B. (2003) The nutritional supplement chromium picolinate generates oxidative DNA damage and peroxidized lipids in vivo. *Polyhedron 22*, 455–463.
[19] Speetjens, J. K., Collins, R. A., Vincent, J. B., and Woski, S. A. (1999) The nutritional supplement chromium (III) tris(picolinate) cleaves DNA. *Chem. Res. Toxicol. 12*, 483–487.
[20] Hepburn, D. D. D., and Vincent, J. B. (2002) In vivo distribution of chromium from chromium picolinate in rats and implications for the safety of the dietary supplement. *Chem. Res. Toxicol. 15*, 93–100.
[21] Bagchi, D., Stohs, S. J., Downs, B. W., Bagchi, M., and Preuss, H. G. (2002) Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology 180*, 5–22.

# Index

This page intentionally left blank